ACTA UNIVERSITATIS SZEGEDIENSIS

ACTA BIOLOGICA

NOVA SERIES

TOMUS XXX FASCICULI 1—4

SZEGED (HUNGARIA)
1984

ACTA UNIVERSITATIS SZEGEDIENSIS

ACTA BIOLOGICA

NOVA SERIES

TOMUS XXX FASCICULI 1—4

SZEGED (HUNGARIA)
1984

Kiadja

a Szegedi József Attila Tudományegyetem Természettudományi Kara
(Szeged, Aradi vértanúk tere 1)

ISSN 0563—0592

Kiadványunk rövidítése
Acta Biol. Szeged.

Acta Biol. Szeged. 30, pp. 3–10 (1984)

ISOLATION AND CHARACTERIZATION OF THERMOSTABLE ALDOLASE FROM A FACULTATIVE THERMOPHILIC BACTERIUM

MAGDOLNA ÁBRAHÁM and L. BOROSS

Department of Biochemistry, Attila József University, Szeged
(Received June 30, 1983)

Abstract

The aldolase of a facultative thermophilic bacterium, *Bacillus sp.* was characterized. The stability of the enzyme was compared with those of E. coli aldolase and rabbit muscle aldolase. The thermophilic enzyme belongs to class II of D-fructose-1.6-diphosphate aldolases. The thermophilic aldolase proved to be more stable than the mesophile. The substrate and 2-mercaptoethanol increased the stability of the thermophilic aldolase.

Key words: Thermostable, aldolase, characterization.

Introduction

Microorganisms growing at elevated temperatures possess a higher intrinsic thermostability than their mesophilic counterparts. There are several explanations for the thermostability of proteins. The theory of transferable protective factors (KOFFLER, 1957) has no experimental basis. AMELUNXEN and LINS (1968) verified the absence of stabilizing or labilizing factors. In vivo studies have revealed that the thermostability of some enzymes may stem from the interactions of the cellular components (HACHIMORI et al., 1974; WEDLER and HOFFMANN, 1974). Cofactors, substrates and monovalent or divalent ions may result in intracellular thermostability. According to other results, the reason for the thermostability may be the types and number of stabilizing forces such as H-bonds, apolar and ionic interactions operating in the enzyme molecule (CASS and STELLWAGEN, 1975; BRANDTS, 1967).

Thermophilic aldolase from *Bacillus stearothermophilus* has been investigated by JACK and HARRIS (1975). It is a dimeric Zn^{2+}-containing enzyme, with a half-life of about 30 min at 337 K. A gradual loss of activity was observed during the isolation, which could be prevented by adding Co^{2+} ion to the buffers. The Co^{2+}-enzyme cannot be inactivated to any detectable extent during 80 min at 337 K. Our study serves the comparative investigations between the aldolase of a facultative thermophile, *Bacillus sp.* JB-1 and two different types of mesophilic aldolases. The mesophilic enzymes were purified from *E. coli* and rabbit muscle, respectively. The thermophilic aldolase was characterized and the thermal stabilities of the three aldolases and the effects of various cations and substrate on the thermal stability were investigated.

Materials and Methods

Chemicals for general use were of analytical grade and were purchased from Reanal Factory of Laboratory Chemicals (Budapest, Hungary). Lysozyme was purchased from Serva Feinbiochemica (Heidelberg, FRG) and Sephacryl-200 and Sephadex gels from Pharmacia Fine Chemicals (Uppsala, Sweden).

Cultural conditions. A facultative thermophile, *Bacillus sp.* isolated at our Department and *E. coli* C-600 were maintained on 3% agar-agar. The thermophile strain was grown at 323 K with vigorous aeration in a medium containing 0.5% Yeast Extract Oxid, 1% glucose, 0.5% $(NH_4)_2SO_4$, 0.1% KH_2PO_4, 0.05% $MgSO_4 \times 7H_2O$, 0.01% $CaCl_2$. The pH of the medium was adjusted with 0.1 M NaOH to 7.5. Under these conditions the cells reached the end of the log phase of growth within 6 hours.

The *E. coli* cells were grown at 203 K with intensive shaking. The medium contained 1% Bacto Tryptone Difco, 0.5% Yeast Extract Oxoid, 0.5% glucose, and 0.5% NaCl in 0.1 M Tris/HCl buffer (pH 7.3). The cells reached the end of the log phase of growth within 16 hours.

The rabbit muscle aldolase was prepared and recrystallized three times according to TAYLOR et al. (1948). Its specific activity was 10 U/mg (in coupled reaction assay).

Enzyme assay was performed according to the method of JAGANATHAN et al. (1956) or with a coupled reaction assay in the presence of glycerol-3-phosphate dehydrogenase (GDH) and triosephosphate isomerase (TIM) (RUTTER et al., 1966). In the first case the reaction mixture contained 7 µmole hydrazine and 12 µmole D-furctose-1.6-diphosphate (FDP) in 0.1 M phosphate buffer (pH 7.5). Measurements were carried out at 240 nm, at 298 K.

The coupled reaction assays were performed in an assay mixture containing 0.1 mM NADH, 0.22 mM FDP, and 40 µg/ml GDH/TIM [the specific activity of GDH was approx. 130 U/mg in 0.2 M triethanolamine buffer (pH 7.9)], at 298 K.

Protein content of purified enzyme was determined spectrophotometrically. The absorption coefficient was found to be $A^{0.1\%} = 0.62$ for the thermophilic aldolase. The protein content for rabbit muscle aldolase was taken as $A^{0.1\%} = 0.75$ reported by FOX and DANDLIKER (1956).

Thermal inactivation was carried out in 0.1 M phosphate buffer, pH 7.5, as previously described by SZAJÁNI et al., (1970). 1 ml aliquots of enzyme solution with protein contents of 1.0—1.5 mg were incubated at the selected temperatures, then cooled and centrifuged.

Molecular weight was determined on Sephacryl-200 column (1.5×43 cm). Trypsin (Mw 23.800), hemoglobin (Mw 64.000), bovin serum albumin (Mw 68.000) and glucose oxidase (Mw 150.000) were used as molecular weight standards.

Results and discussion

PREPARATION OF BACTERIAL ALDOLASES.

Cells were cooled to 283 K and centrifuged. 50 g cell paste was washed twice with 0.1 M phosphate buffer (pH 7.5), containing 0.9% NaCl. Cells were lysed by the addition of lysozyme (1 mg/g cells). The suspension was stirred in a water-bath for two hours at 310 K, then overnight at 277 K. The lysis was performed too in french press (Aminco) at 20 000 psi cell pressure for 5 min. The degree of enzymatic lysis approached that of second method. Cell debris was removed by centrifugation at 20 000 g for 20 min. The precipitate was washed with the same buffer and the supernatants were collected. The crude extract was saturated up to 0.5 with solid $(NH_4)_2SO_4$ at 298 K, and the precipitate was removed by centrifugation. The supernatant was further saturated up to 0.75 with $(NH_4)_2SO_4$, the precipitate contained high aldolase activity. The protein was separated from the salt on a Sephadex G-25 column (2.5×20 cm) which was equilibrated with 0.02 M phosphate buffer (pH 7.5). The active fractions were collected and passed through a DEAE-cellulose column (1.5×11 cm) in 0.02 M phosphate buffer (pH. 7.5). The enzyme was eluted with the same buffer containing 0.2 M NaCl. The active fractions were collected and gel-filtered on a Sephadex G-150 column (2.5×30 cm) equilibrated with 0.1 M phos-

phate buffer (pH 7.5). The buffer contained 1 mM 2-mercaptoethanol in the case of the thermophilic enzyme, for without it the purified enzyme lost much of its activity during several hours.

CHARACTERIZATION OF THE THERMOPHILIC ALDOLASE.

The activity of the thermophilic aldolase has an optimum at 337 K and pH 7.5 (Fig. 1; 2). The specific activity was calculated as 0.32 unit/mg; the value of K_M was 7.3×10^{-4} M (Fig. 3).

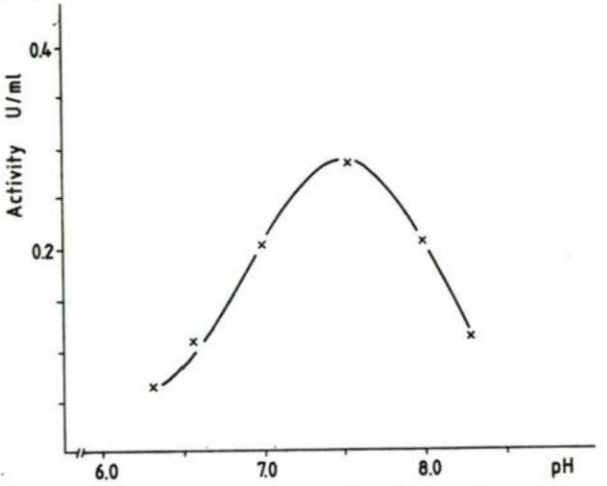


Fig. 1. Effect of pH on the activity of the thermophilic aldolase. The measurements were carried out in 0.1 M phosphate buffer with 0.1 mg/ml enzyme at 330 K.

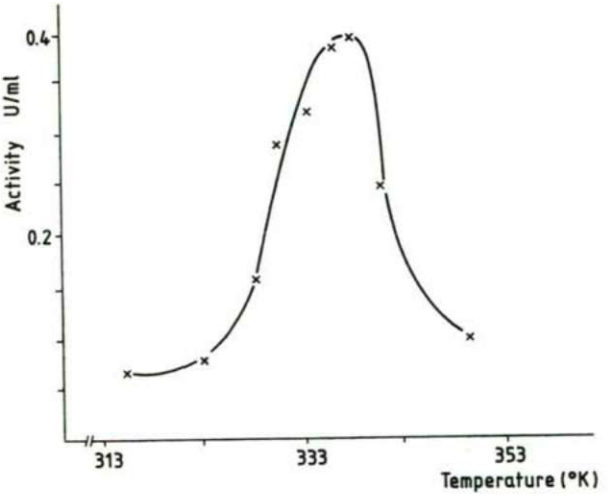


Fig. 2. Effect of temperature on the activity of the thermophilic aldolase. The measurements were carried out in 0.1 M phosphate buffer (pH 7.5) with 0.1 mg/ml enzyme.

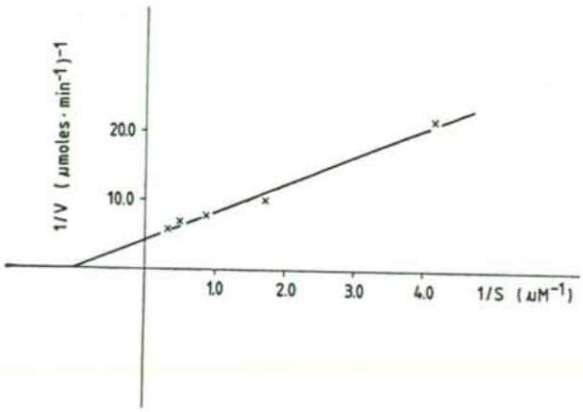


Fig. 3. Lineweaver — Burke plot for thermostable aldolase. Experiments were carried out in 0.2 M triethanolamine buffer (pH 7.9), with coupled reaction enzyme assay, at 298 K.

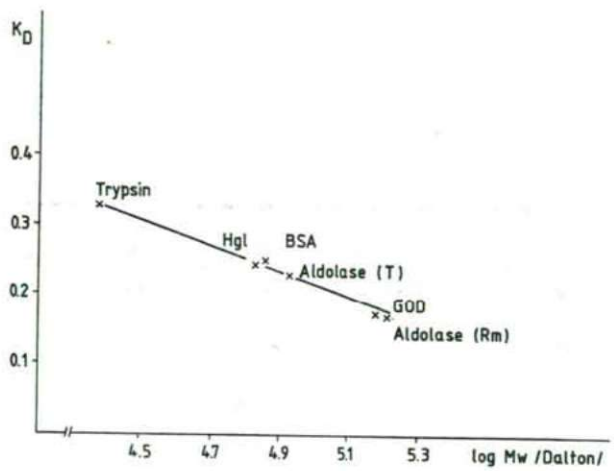


Fig. 4. Determination of molecular weight of the thermophilic aldolase on Sephacryl-200 column (1.5×43 cm.) The proteins were dissolved in 0.1 M Tris/HCl buffer (pH 7.5), containing 0.2 M NaCl. Standard proteins used were: trypsin, hemoglobin (Hgl), bovine serum albumin (BSA), rabbit muscle aldolase, glucose oxidase (GOD).

The molecular weight of the thermophilic enzyme was determined as 80. 300 dalton which is in good agreement with that of bacterial aldolases (Fig. 4).

The activity of the enzyme was not significantly enhanced by Zn^{2+}, Mg^{2+}, Na^{+} or K^{+} ions, but Co^{2+}, Ni^{2+}, Ca^{2+}, Mn^{2+} ions in 1.0 mM concentration increased the activity (Table I).

The effect of the Co^{2+} ion concentration on the activity of the thermophilic aldolase is shown in the Table II. The highest activity was measured at 1.0 mM $CoCl_2$. Considerable loss of activity occurred on the action of EDTA (Table III). This was

reversible, by the effect of 10 mM EDTA the enzyme was inhibited to 100%, but 80% of the activity was restored by adding 10 mM $CoCl_2$.

Thermal inactivation tests were carried out on the thermophilic and mesophilic aldolases.

Table 1. Effects of different cations on the activity of the thermophilic aldolase. Enzyme assays were performed in 0.1 M Tris/HCl buffer, pH 7.5. The enzyme concentration in the reaction mixture was 0.16 mg/ml.

Ions	Relative activity % Ion concentration	
	0.2 mM	1.0 mM
Ni^{2+}	89.4	141
Zn^{2+}	97.6	82.3
Ca^{2+}	105.7	135
Mg^{2+}	105.7	70.6
Mn^{2+}	81.3	117.7
Co^{2+}	181.3	370
Na^{+}	105.7	100
K^{+}	105.7	88.2

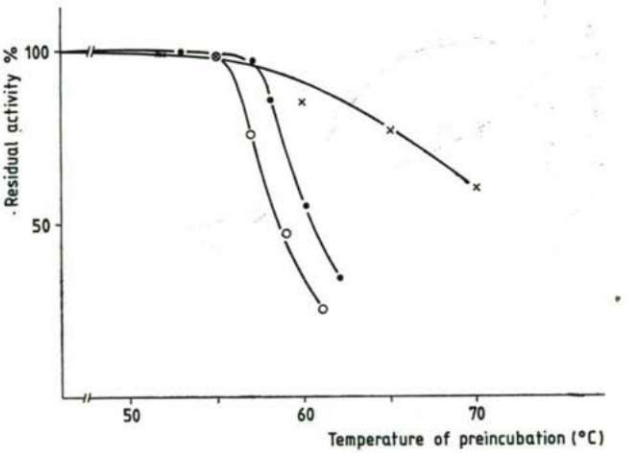


Fig. 5. Heat inactivation of *E. coli* aldolase (○——○) rabbit muscle aldolase (●——●) and the thermophilic aldolase (x——x). The samples were incubated at various temperatures for 10 minutes in 0.1 M phosphate buffer (pH 7.5). The protein content was 1.1 mg/ml.

A comparison of the thermal stabilities of *E. coli* aldolase, rabbit muscle aldolase and the thermophilic aldolase is shown in Fig. 5.

The aldolases from the mesophiles had similar thermal stabilities and their half-lives were comparable in the same range of temperature. When the protein

solution was incubated at 331 K in 0.1 M phosphate buffer (pH 7.5), the activity of muscle aldolase decreased to 50% during about 60 min, which is in good agreement with the data of ZÁVODSZKY et al. (1972).

Table 2. Effect of Co^{2+} ion concentration on the activity of thermophilic aldolase. The enzyme activity was measured in 0.1 M Tris/HCl buffer (pH 7.5) with an aldolase concentration of 0.16 mg/ml at 298 K.

Co^{2+} ion concentration M	Relative activity %
0.0	100
1×10^{-5}	155.1
2×10^{-5}	180.7
1×10^{-4}	251.8
2×10^{-4}	265.1
1×10^{-3}	346.1
2×10^{-3}	322.0

The thermal stability of the thermophilic aldolase highly saturated with FDP was modified by the substrate and the same effect was observed incubating the enzyme in 2-mercaptoethanol at 333 K (Fig. 6). These compounds markedly enhanced the stability of the enzyme.

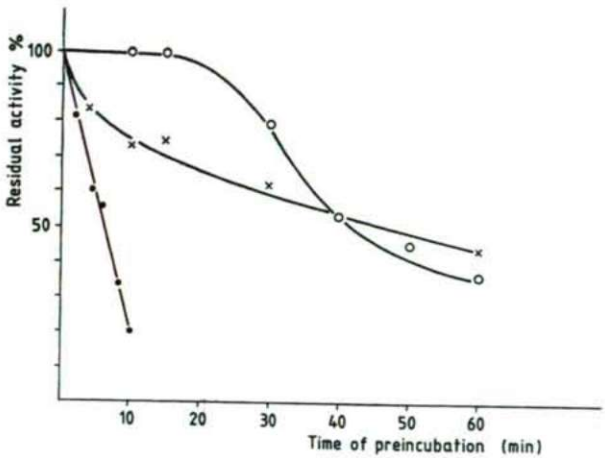


Fig. 6. Effects of substrate and 2-mercaptoethanol on the thermal stability of the thermophilic aldolase at 333 K. Heat treatment was performed in 0.1 M phosphate buffer (pH 7.5), containing 1.2 mM D-fructose-1.6-diphosphate (○——○) or 1 mM 2-mercaptoethanol (x——x). In the control samples the substrate and reducing compound were omitted from the protein solution (●——●). The enzyme concentration was 1.25 mg/ml.

To summarise the results, the thermophilic aldolase isolated from a facultative thermophile, *Bacillus sp.*, has a pH optimum of 7.5 at 337 K, the optimum temperature. Co^{2+} ions increased the activity. The aldolase was inhibited by EDTA. The ion

Table 3. Effect of EDTA on the enzymatic activity of the thermophilic aldolase. The activity was measured in 0.1 M Tris/HCl buffer (pH. 7.5) at 298 K. The enzyme concentration was 0.16 mg/ml.

Final molar of EDTA M	Relative activity %
0.0	100
3.5×10^{-4}	73.5
5.5×10^{-4}	48.6
1.0×10^{-3}	30.1
1.0×10^{-2}	0.0

dependence of the enzyme activity suggests that the thermophilic aldolase belongs to class II of D-fructose-1.6-diphosphate aldolases. Although this enzyme is similar to the aldolase of *Bacillus stearothermophilus* reported by JACK and HARRIS (1975), which also shows a high affinity towards Co^{2+} ions, addition of Zn^{2+} ions did not increase the activity in our case. The thermal stability of the thermophilic enzyme was higher than that of the mesophiles. The thermal stabilities of *E. coli* aldolase and muscle aldolase did not differ significantly from each other. The thermophilic enzyme requires thiol compounds for stability, whereas the aldolase from *E. coli* has no such requirement (BALDWIN et al., 1978). The stabilizing effect of the thermophilic enzyme has been observed. A general assumption concerning the thermal stability is that the substrate and thiol compounds conserve or convert the enzyme to a thermally stable and active conformation.

References

AMELUNXEN, R. E. and LINS, M. (1968): Comparative thermostability of enzymes from *Bacillus stearothermophilus* and *Bacillus cereus*. — Arch. Biochem. Biophys. *125*, 765–769.

BALDWIN, S. A., PERHAM, R. N. and STRIBLING, D. (1978): Purification and characterisation of the class-II-fructose 1.6-bisphosphate aldolase from *Escherichia coli* (Crookes' strain). — Biochem. J. *169*, 643–652.

BRANDTS, J. F. (1967): Thermobiology. Acad. Press, New York—San Francisco—London.

CASS, K. H. and STELLWAGEN, E. (1975): Thermostable phosphofructokinase from the extreme Thermophile Thermus ×-1. — Arch. Biochem. Biophys. *171*, 682–694.

FOX, J. B. and DANDLIKER, W. B. (1956): Study of the physical properties of glyceraldehyde-3-phosphate dehydrogenase. — J. Biol. Chem. *218*, 53–57.

HACHIMORI, A., MATSUNAGA, A., SHIMIZU, M., SAMEJIMA, T. and NOSOH, Y. (1974): Purification and properties of glutamine synthetase from *Bacillus stearothermophilus*. — Biochim. Biophys. Acta *350*, 461–474.

JACK, R. and HARRIS, J. I. (1975): Enzymes and proteins from thermophilic microorganisms. Proc. International Symp., Zurich. Birkhäuser Verlag, Basel und Stuttgart.

JAGANATHAN, V., SINGH, K. and DAMODARAN, M. (1956): Carbohydrate metabolism in citric acid fermentation. — Biochem. J. *63*, 94–105.

KOFFLER, H. (1957): Protoplasmic differences between mesophiles and thermophiles. — Bacteriol. Rev. *21*, 227–240.

RUTTER, W. J., HUNSLEY, J. R., GROVES, W. E., CALDER, J., RAJKUMAR, T. V. and WOODFIN, B. M (1966): Fructose diphosphate aldolase. In: Colowick, S. P. and Kaplan, N. O. (eds.), Method in enzymology, IX. — Acad. Press, New York—London.

SHAPIRO, S., ENSER, M., PUGH, E. and HORECKER, B. L. (1968): The effect of pyridoxal phosphate on rabbit muscle aldolase. — Arch. Biochem. Biophys. *128*, 554–561.

SZAJÁNI, B., SAJGÓ, M., BISZKU, E., FRIEDRICH, P. and SZABOLCSI, G. (1970): Identification of a cysteinyl residue involved in the activity of rabbit muscle aldolase. — Eur. J. Biochem. *15*, 171–178.

TAYLOR, J. F., GREEN, A. A. and CORI, G. T. (1948): Crystalline aldolase. — J. Biol. Chem. *173*, 591–604.

Address of the authors
DR. MAGDOLNA ÁBRAHÁM
DR. L. BOROSS
Department of Biochemistry
Attila József University
Szeged, P.O. Box 533
H-6701

Acta Biol. Szeged. 30, pp. 11–17 (1984)

EFFECTS OF HERBICIDES ON THE CYTOCHROME P-450 CONTENT OF LIVER MICROSOMES IN CARP (CYPRINUS CARPIO L.)

L. MARIA SIMON, L. BOROSS and J. NEMCSÓK

Department of Biochemistry, Attila József University, Szeged

(Received June 30, 1983)

Abstract

Ultracide (methidathion), paraquat and $CuSO_4$ were administered in 0.5–10 ppm concentrations to carp and the cytochrome P–450 contents of the liver microsomes were determined. After treatment with $CuSO_4$ the cytochrome P–450 content increased. For paraquat and ultracide, decreases were observed after several days of administration. Alterations in cytochrome P–450 levels might be due either to direct action of the herbicides or to changes caused by them in the membrane structure of the endoplasmic reticulum.

Key-words: herbicides, cytochrome P–450, carp.

Introduction

The extensive use of herbicides has increased the incidence of environmental pollution. Herbicides are among the most dangerous agents of water contamination, since they are used on or near the soil and in many instances in water for aquatic weed control. One property of many biologically active compounds such as drugs or pesticides is their ability to induce the microsomal enzyme systems of animals (CONNEY, 1967; MANNERING, 1968). Recent evidence has shown that the enzyme induction following herbicide treatment is the most sensitive parameter among the biological and toxic responses in mammals (CARLSON and SCHOENIG, 1980). Cytochrome P-450, the carbon monoxide binding pigment of microsomes, serves as the terminal oxidase in the metabolism of a wide variety of substrates, including drugs, insecticides, chemical carcinogens, fatty acids and steroids (MANNERING et al., 1969). Two techniques frequently used to determine the potential for agents to induce microsomal enzyme systems are quantitative analysis of the haemoprotein cytochrome P-450 and measurement of the NADPH cytochrome c reductase activity. In its reduced form, cytochrome P-450 complexes with carbon monoxide and the complex exhibits an absorption maximum at 450 nm (ALVARES et al., 1967). Various chemicals have different effects on the quantity or activity of this cytochrome and these measurements can usually be employed to characterize the agents.

In an early report, BRODIE and MAICKEL (1962) suggested that microsomal drugmetabolizing enzymes might be absent in fish. Subsequent studies, however, have demonstrated the presence of various levels of oxidative drugmetabolizing activity in some vertebrate species of fresh water and marine origin, and also in insects. CREAVEN et al. (1967) concluded that microsomal drug-metabolizing enzymes do occur in trout and amphibia but at lower levels than in mammals. The presence

of cytochrome P-450 in the microsomal fraction of trout liver was reported by CHAN et al. (1967).

The purpose of the present study was to characterize the effects of three kinds of herbicides, paraquat, $CuSO_4$ and ultracide, on the cytochrome P-450 content of carp liver.

Materials and methods

Chemicals: Paraquat (1,1' -dimethyl-4,4 -bipyridylium dichloride) was purchased as Gramoxone (ICI Plant Protection Division, England), which contains 24% of the herbicide in aqueous solution. Methidathion (0,0-dimethyl-S-2-methoxy-1,3, 4-thiadiazol-5(4H)-yl-4-methyl-dithiophosphate) was obtained as ultracide (Ciba-Geigy A. G.). All other chemicals were of analytical grade and were obtained from Reanal, Hungary.

Common carp (*Cyprinus carpio* L.) weighing 600–800 g were obtained from the Fisheries Research Institute in Szarvas, Hungary, and were acclimatised for at least 5–7 days before treatment. Herbicides were administered to the water of the fish in 0.5–10 ppm concentrations. (The concentrations of herbicides were calculated on the basis of effective substances.) Carps were killed and the livers were removed. The livers were minced in ice-cold 50 mM Tris HCl buffer, pH 7.4 containing 0.25 M sucrose and homogenized to a final homogenate concentration of 25% (w/v) using a glass Potter-Elvehjem homogenizer with a teflon pestle. Microsomes were prepared according to KAMATH and NARAYAN (1972). Protein content was measured by the method of LOWRY et al. (1951). Cytochrome P–450 was estimated from the absorption difference at 450 and 490 nm, by the application of an extinction coefficient of 91 mM^{-1} (OMURA and SATO, 1964). The difference spectra were recorded using a Pye Unicam SP spectrophotometer. The given values are the averages ±(S. D.) for 3–5 fish specimens.

Results

EFFECT OF PARAQUAT

In our experiments paraquat was applied in concentrations of from 0.5 to 7 ppm. After 24 hours of treatment the cytochrome P-450 content of the liver microsomes was increased (Fig. 1). At a 0.5 ppm herbicide concentration the increase was 10%.

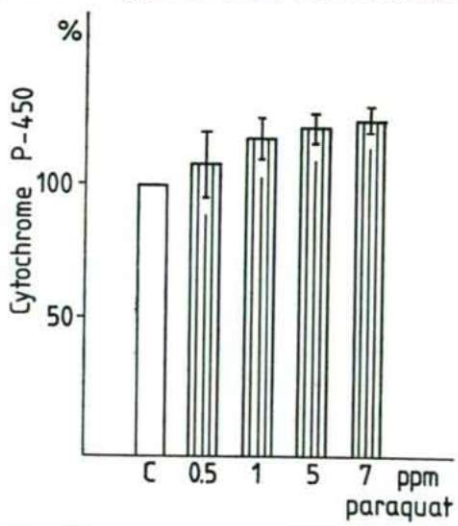


Fig. 1. Effect of paraquat in different concentrations on cytochrome P–450 content of liver. The values are averages of those measured from 3–5 individuals as expressed in the percentage of the controls.

With the higher concentration of paraquat the cytochrome P-450 content of the microsomes was about 25% higher than in the control fish. This increase in haemoprotein content, however, was not permanent and during longer treatment a secondary decrease was found. The time curve of the concentration of cytochrome P-450 in the

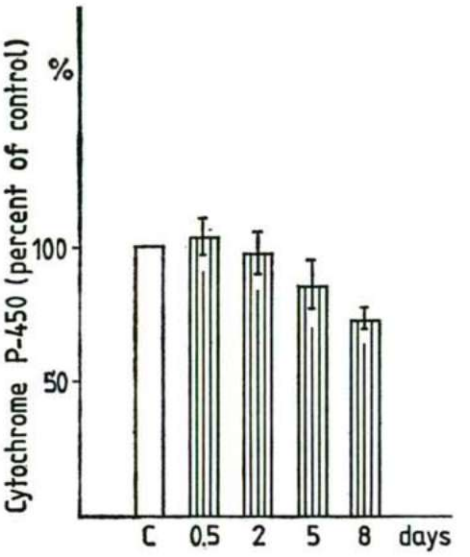


Fig. 2. Effect of 0.5 ppm paraquat on the level of cytochrome P-450 of carp liver microsomes. The values are averages of those measured from 3–5 individuals and expressed in the percentage of the controls.

liver of fish treated with 0.5 ppm paraquat is shown in Fig. 2. It can be seen that on the second day the level of this protein is roughly equal to the original (i. e. untreated) value. During longer treatment with paraquat a significant decrease in the cytochrome P-450 content was observed.

On the eighth day only 72% of the original value was present in the treated fish.

Effect of copper sulphate

During the treatment $CuSO_4$ was used in 3, 5 and 7 ppm concentrations. After 24 hours of administration the cytochrome P-450 level of the liver microsomes was increased (Fig. 3). At 5 ppm concentration an increase of about 35% was measured in the treated carp as compared to the controls. A further increase in the concentration of $CuSO_4$ apparently had no further enhancing effect.

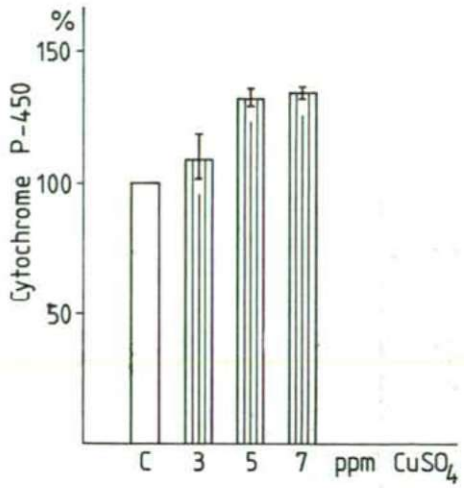


Fig. 3. Changes in the cytochrome P–450 content of liver after $CuSO_4$ treatment in vivo.

In another experiment the effect of 0.5 ppm of $CuSO_4$ was studied during a longer period. The level of cytochrome P-450 on the first day, of treatment was practically the same as in the untreated controls. However, on the second day the value was about 20% higher and this value was also detected on the third day (Fig. 4).

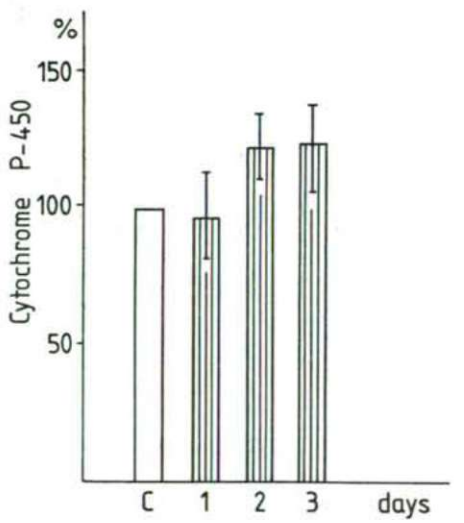


Fig. 4. The action of 0.5 ppm $CuSO_4$ on the level of cytochrome P-450 of carp liver after 24 hours of treatment.

EFFECT OF ULTRACIDE

Changes induced in the cytochrome P-450 concentration of carp liver by ultracide are shown in Fig. 5. After one day, the applied doses of this compound caused increases in the cytochrome P-450 level compared to the control values. After three and four days of ultracide treatment significant decreases were found in the cytochrome

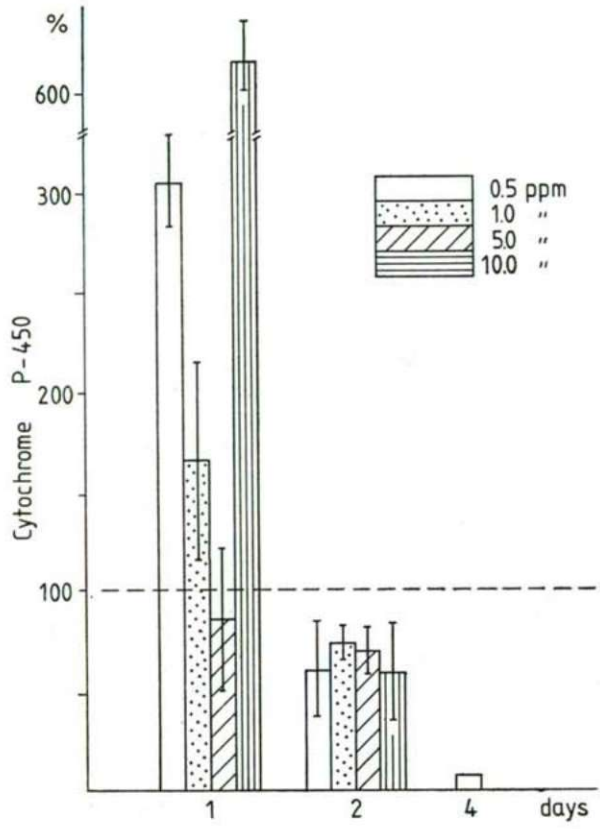


Fig. 5. Effect of ultracide in different concentrations on cytochrome P–450 of liver. The values are averages of those measured from 3–5 individuals and expressed in the percentage of the controls.

P-450 concentration. On the third day the cytochrome P-450 level was decreased to 75—80% as compared to the controls for all applied concentrations. After four days of treatment the presence of cytochrome P-450 could be shown only in fish treated with 0.5 ppm of herbicide, and this value was about 7% of the original. A higher concentration of the herbicide resulted in the total loss of this haemoprotein.

Discussion

Recently many reports have been published on the mechanism of paraquat action (MONTGOMERY, 1976; STEFFEN et al., 1980). Paraquat may alter the lipid metabolism and DNA and protein synthesis in the lung of rats. In an earlier paper we reported that paraquat could affect the glycogen metabolism of the liver in fish (SIMON et al., 1982). NEMCSOK et al. (1981) found morphological alterations in the liver of fish after paraquat treatment. Our results suggest that changes in the cytochrome P-450 level of liver treated with paraquat might be due to damage caused by this herbicide in the endoplasmic reticulum membrane.

As a result of studies in recent years it has become apparent that fish and other aquatic organisms are able to accumulate and retain trace elements from their environment. For both fish and mammals the liver is known to be an organ of considerable importance in the storage and uptake of metals (O'DELL and CAMPBELL, 1970) and it is also known to be the site of a number of detoxification functions. Metals can alter the enzyme activities of the liver. Lead, for example, is an inhibitor of the synthesis of haeme in the liver, inhibiting 5-aminolaevulinate dehydratase (SCOPPA et al., 1973).

Our results suggest that herbicides probably affect the synthesis of haeme in the liver or decrease the haeme oxidase activity, diminishing the turnover of haeme in the liver.

Some agents are known to decrease the affinity of the apoprotein of cytochrome P—450 for haeme, so that some of the haeme present bound in cytochrome P—450 at the time of poisoning will subsequently be released and made available for degradation (JÄRVISALO et al., 1978; BISSEL and HAMMAKER, 1976). It is possible that methidathion acts in the same way as the agents mentioned above.

Our results show that the applied herbicides can alter the cytochrome P-450 content of carp liver. Cytochrome P-450 is known to play an important role in the hydroxylation of various xenobiotics in the organism. Thus, every change in its concentration may result in a change of the liver's ability to metabolize xenobiotics in the body. However, it can not yet be stated for sure what causes the changes in cytochrome P-450 concentration. It is expected that future investigations will provide an answer to this question.

Acknowledgements

The authors would like to thank DR. JÁNOS OLÁH, Scientific Director of the Fisheries Research Institute of Szarvas for kind help in supplying the carp used in this work. They also gratefully acknowledge the excellent technical assistance of Mrs. B. ÁGNES BALÁZS.

References

ALVARES, A. P., SCHILLING, G., LEVIN, W. and KUNTZMANN, R. (1967): Studies on the induction of CO-binding pigments in liver microsomes by phenobarbital and 3-methylcholanthrene. — -Biochem. Biophys. Res. Commun. *39*, 521–526.

BISSEL, D. M. and HAMMAKER, L. E. (1976): Cytochrome P–450 heme and the regulation of hepatic heme oxigenase activity. — Arch. Biochem. Biophys. *176*, 91–102.

BRODIE, B. B. and MAICKEL, R. P. (1962): Comparative biochemistry of drug metabolism. — In: Proc. 1st Internat. Pharmacol. Meeting *6*, 299. Pergamon Press, London.

CARLSON, G. P. and SHOENIG, G. P. (1980): Induction of liver microsomal NADPH cytochrome c reductase and cytochrome P–450 by some new synthetic pyrethroids. — Toxicol. Appl. Pharm. *52*, 507–512.

CHAN, T. M., GILLET, J. W. and TERRIERE, L. C. (1967): Interaction between microsomal electron transport systems of trout and male rat by cyclodiene epoxidation. — Comp. Biochem. Physiol. *20*, 731–742.

CONNEY, A. H. (1967): Pharmacological implications of microsomal enzyme induction. — Pharmacol. Rev. *19*, 317–366.

CREAVEN, P. J., DAVIS, W. H. and WILLIAMS, R. T. (1967): Dealkylation of alkoxybiphenyls by trout and frog liver preparations. — Life Sci. *6*, 105–111.

JÄRVISALO, J., GIBBS, A. H. and DE MATTEIS, F. (1978): Accelerated conversion of heme to bile pigments caused in the liver by carbon disulfide and other sulfur-containing chemicals. — Mol. Pharmacol. *14*, 1099–1106.

KAMATH, S. A. and NARAYAN, K. A. (1972): Interaction of Ca^{2+} with endoplasmic reticulum of rat liver: A standardized procedure for the isolation of rat liver microsomes. — Anal. Biochem. *48*, 53–61.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. and RANDALL, R. J. (1951): Protein measurement with the Folin Phenol reagent. — J. Biol. Chem. *193*, 265–268.

MANNERING, G. J. (1968): Significance of stimulation and inhibition of drug metabolism. In Selected Pharmacologic Testing Methods. A. Burger edition, New York, 51–119.

MANNERING, G. J., SLADEK, N. E., PARLI, C. J. and D. W. SHOEMAN, (1969): Formation of a new P–450 hemoprotein after treatment of rats with polycyclic hydrocarbons. In Microsomes and Drug Oxidations. Academic Press, New York, 303–330.

MONTGOMERY M. R. and NIEWOEHNER, D. E. (1976): Oxidant-induced alterations in pulmonary microsomal mixed-function oxidation: Acute effects of paraquat and ozone. — J. Environ. Sci. Health, *3*, 205–219.

NEMCSÓK, J., BENEDECZKY, I., BOROSS, L., ASZTALOS, B. and ORBÁN, L. (1981): Subcellular localization of transaminase enzymes in fishes and their significance in the detection of water pollution. — Acta Biol. Szeged *27*, 9–15.

O'DELL, B. L. and CAMPBELL, B. J. (1971): Trace elements: metabolism and metabolic function. In Comprehensive Biochemistry. Elsevier, New York. *21*, 179–226.

OMURA, T. and SATO, R. (1964): The carbon monoxide-binding pigment of liver microsomes. — J. Biol. Chem. *239*, 2379–2385.

SCOPPA, P., ROUMENGOUS, M. and PENNING, W. (1973): Hepatic drug metabolizing activity in lead-poisoned rats. — Experientia *29*, 970–972.

SIMON, L. M., NEMCSÓK, J. and BOROSS, L. (1983): Studies on the effect of paraquat on glycogen mobilization in liver of common carp *Cyprinus carpio* L. — Comp. Biochem. Physiol. *75C*, 167–169.

STEFFEN, C., MULIAWAN, H. and KAPPU, H. 1980): Lack of in vivo lipid peroxidation in experimental paraquat poisoning. — Naunyn-Schmiedeberg's Arch. Pharmacol. *310*, 241–243.

Address of the authors
DR. L. MÁRIA SIMON, DR. J. NEMCSÓK
and PROF. DR. L. BOROSS
Department of Biochemistry
A. J. University, H-6701 Szeged,
P.O. Box 533, Hungary

Acta Biol. Szeged. 30, pp. 19–25 (1984)

EFFECTS OF 2,4-D AND ATRAZINE ON K AND P UPTAKE BY MAIZE AT DIFFERENT pH VALUES

E. HAUNOLD and F. ZSOLDOS

Institute of Agriculture, Austrian Research Centre, Seibersdorf and Department of Plant Physiology, Attila József University, Szeged

(Received July 30, 1983)

Abstract

The uptakes of the auxin-type herbicide 2,4–D and the non-auxin-type atrazine into maize seedlings (*Zea mays* L. *cv. Funk 350*) and their effects on the potassium and phosphate uptake, were investigated at different pH values. Short incubation of the roots in 0.01 mM 2,4-D caused a marked ion uptake inhibition only at low pH. The non-auxin-type herbicide atrazine did not produce such an inhibitory effect. Lowering the pH of the uptake solution led to an increased 2,4-D influx by the roots, thereby inhibiting the active ion uptake. In the case of atrazine, the change of the pH had no influence on either the herbicide or the ion influx.

Key-words: Auxin-type herbicide, ^{14}C-atrazine, ^{14}C-2,4-D, Cherenkov radiation, Conviron growth chamber, Funk 350, γ-spectrometry, Low pH, Non-auxin-type herbicide, Nutrient solution, ^{32}P, Roots, ^{86}Rb, *Zea mays* L.

Introduction

In the intensive maize production, atrazine, a non-auxin-type herbicide, is widely used for weed control. However, it does not kill all the weeds. After several years of application some weeds, such as *Convolvulus* sps. and *Cirsium* sps., can be spread to an extent which necessitates the use of 2,4-D, an auxin-type herbicide which is very effective against these species (AMMON and HERI, 1979).

The chlorotriazines (i. e. atrazine, simazine, etc.) are readily taken up by the roots of plants, regardless of whether they are resistant or susceptible. In experiments lasting only a few hours, atrazine rapidly permeated potato tuber slices and excised velvetleaf roots (PETERSON and EDGINGTON, 1976; PRICE and BALKE, 1982). Because of this root uptake, chlorotriazines are used for preemergent treatment. The resistant corn roots, however, contain an active compound capable to inactivate atrazine and simazine (KEARNEY and KAUFMAN, 1975).

The phenoxy herbicides (i. e. 2,4-D, MCPA) showing auxin characteristics are also readily absorbed by roots (SCOTT and MORRIS, 1970; ZSOLDOS and HAUNOLD, 1982). In a concentration of 10^{-4} M 2,4-D effectively stops cation uptake by wheat and rice (HAUNOLD and ZSOLDOS, 1976; ZSOLDOS and HAUNOLD, 1979). It was recently found that the bio-activities of auxin compounds vary with the pH of the outer medium (KENNEDY and STEWART, 1980; ZSOLDOS et al., 1978).

Materials and methods

Maize seedlings (*Zea mays* L. *cv. Funk 350*) were used throughout the experiments. To raise the plants, seeds were soaked in running tap water for 3 hours and left to germinate on wet filter paper in large Petri dishes. After 2 days the young seedlings were placed on stainless steel screens and transferred to a growth chamber (CONVIRON–EF 7) or a controlled growth room and cultivated in a nutrient solution containing 5×10^{-4} $CaSO_4$, 10^{-4} M NH_4NO_3 and 10^{-4} M NaH_2PO_4. Intact plants were used at the age of 8 to 14 days. There were usually 5 plants on a screen. To start an experiment, the screens with plants were placed on beakers containing 250 ml uptake solution. The uptake solution contained the ions and the herbicides under study; the concentrations are given in the Figures.

The nutrients and herbicides were labelled (10 μCi ^{86}Rb/1, 10 μCi ^{32}P/1, and 10 μCi ^{14}C/1). After the uptake period the roots were rinsed three times in 400 ml distilled water for 1 min and their activities were then determined (Zsoldos, 1971). Postexperimental washing in inactive (1 mM KCl + +0.5 mM $CaCl_2$) solution (5 min) was also performed to remove ions from the free space. It was found that the activity in the roots decreased by only 6–8% compared to washing for 1 min in distilled water. In our case this difference had no special importance and the experimental material was therefore washed in distilled water.

^{86}Rb activity was measured directly in the roots by γ-spectrometry. For ^{32}P measurement Cherenkov radiation was used. ^{14}C was measured by LSC after the roots had been burned in a Packard sample oxidizer. All experiments were carried out on 3 parallel samples; the data given below are the averages. The deviations between the data from the individual determinations were within 10%.

Results

The uptake of K(^{86}Rb) by intact maize roots increased with time and pH (Fig. 1.). In the presence of 2.4-D (0.01 mM) the uptake of K was reduced, the reduction being stronger at lower pH. When atrazine (0.01 mM) was present (Fig. 2.), the uptake of K was not impeded and increased both with time and pH.

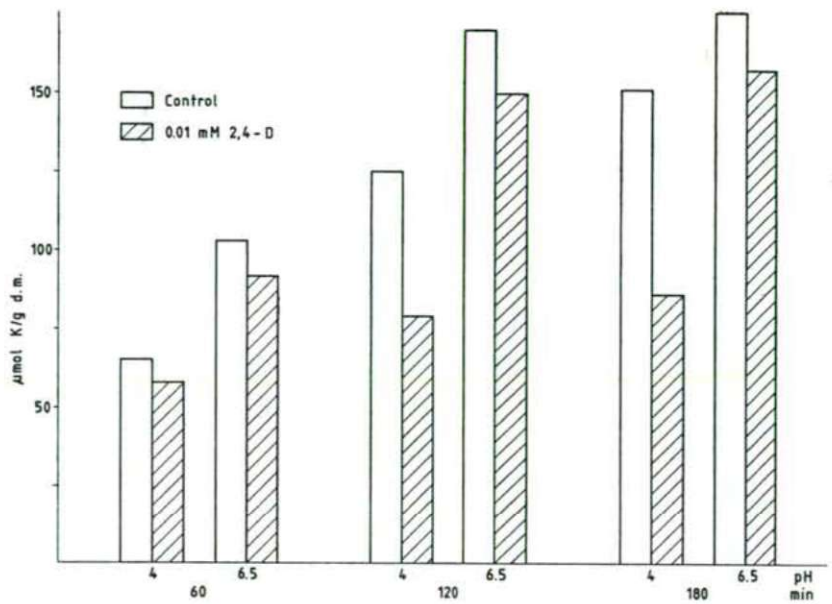


Fig. 1. Effects of 2,4-D on K(^{86}Rb) uptake by maize roots at pH 4 and 6.5. The uptake solution contained 5×10^{-4} M $CaSO_4$, 10^{-4} M $MgSO_4$, 10^{-4} M NH_4NO_3 and 10^{-4} M NaH_2PO_4.

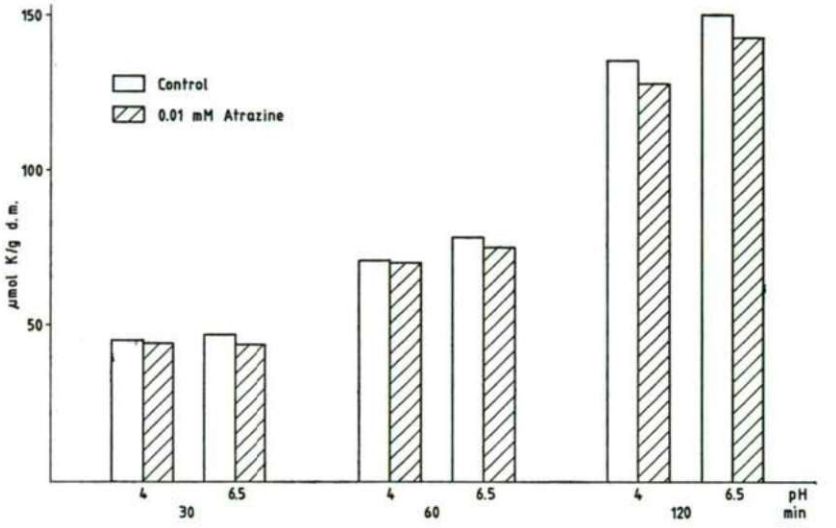


Fig. 2. Effects of atrazine on K(^{86}Rb) uptake by maize roots at pH 4 and 6.5. Otherwise as in Fig. 1.

The P uptake increased with time and the decrease of pH. In the presence of 2,4-D (0.01 mM) the uptake of P was reduced at both pH values, but relatively more at 4 than at 6.5 as compared to the control (Fig. 3). Atrazine (0.01 mM) did not exert much influence on the P uptake; the P uptake increased with time and decreased with increasing pH (Fig. 4).

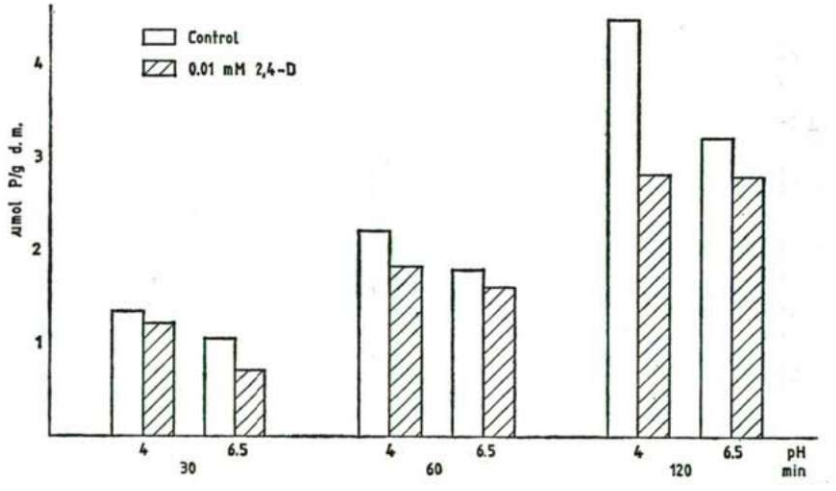


Fig. 3. Effects of 2,4-D on P uptake by maize roots at pH 4 and 6.5. The uptake solution contained 5×10^{-4} M $CaSO_4$, 10^{-4} M $NH_4H_2PO_4$, 10^{-4} M KNO_3 and 10^{-4} M $MgCl_2$.

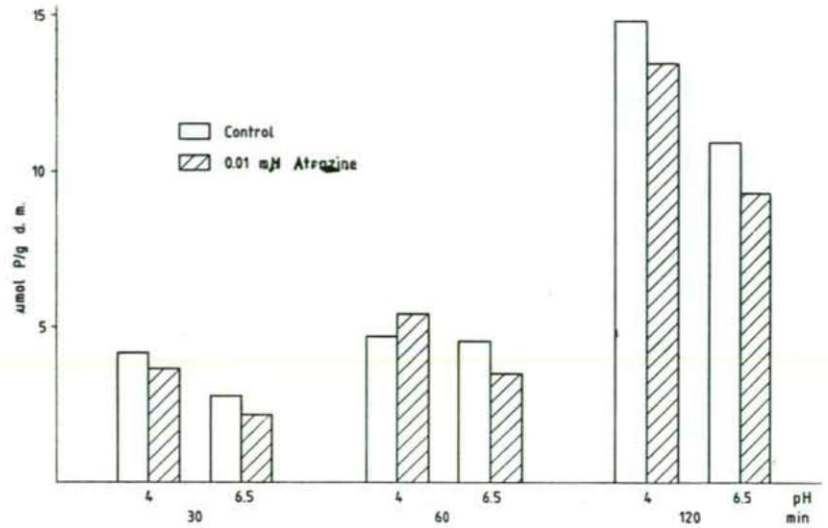


Fig. 4. Effects of atrazine on P uptake by maize roots at pH 4 and 6.5. Otherwise as in Fig. 3.

The uptake of 2.4-D (0.01 mM) by maize roots increased with time and was higher by a factor of 20 at pH 4 than at 6.5. Only 1/700—1/500 of the amount taken up at pH 4 was translocated; the amount translocated increased with time. At pH 6.5 the translocation amounted to 1/600—1/300 of the uptake (Fig. 5).

The uptake of atrazine (0.01 mM) increased with time and was of similar magni-

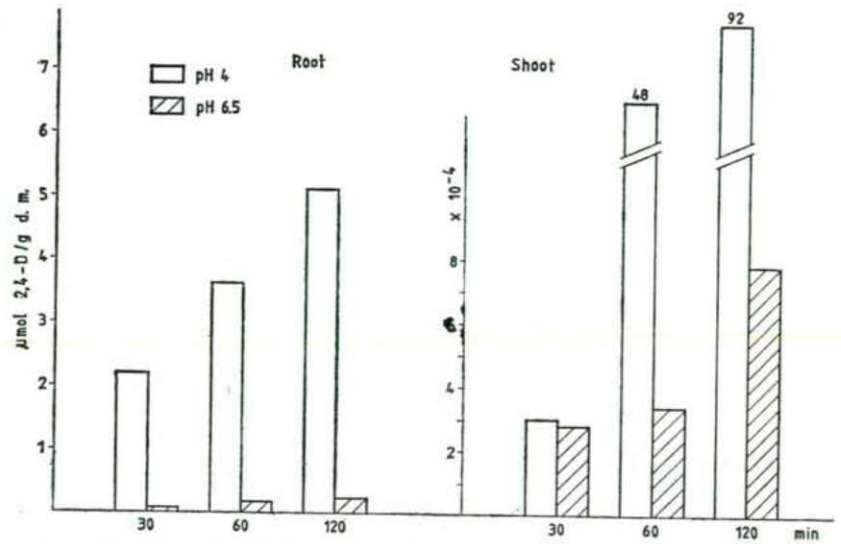


Fig. 5. Effects of pH on uptake of ^{14}C-2,4-D and its transport within maize plants, The uptake solution contained 0.01 mM 2,4-D + 5×10^{-4} M $CaSO_4$ + 10^{-3} M KCl + 10^{-4} M $MgCl_2$ and 10^{-4} M NaH_2PO_4.

tude at the two pH values. Compared to 2,4-D, the atrazine uptake at pH 4 was 30 times and at pH 6.5 2 times lower. Of the atrazine taken up at pH 4, 1/160—1/80 was translocated, compared with 1/120—1/30 at pH 6.5 (Fig. 6).

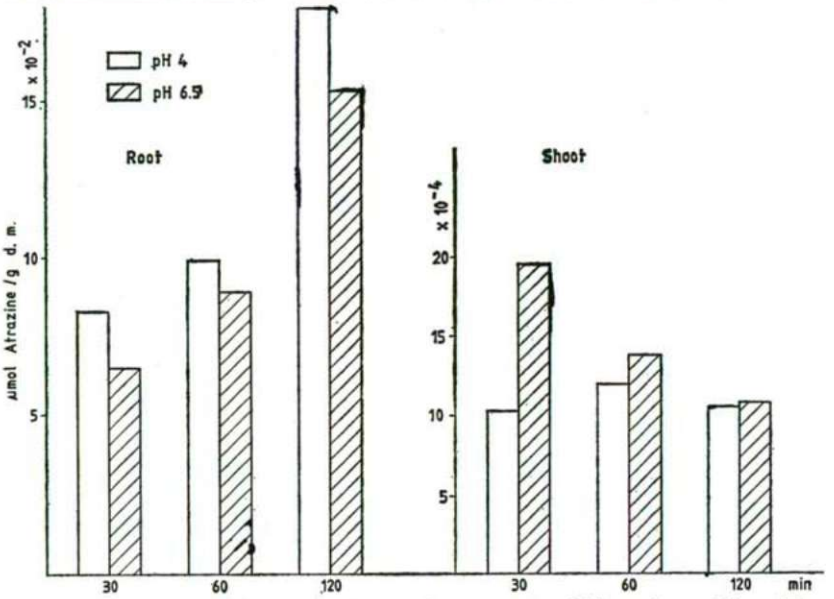


Fig. 6. Effects of pH on uptake of ^{14}C-atrazine and its transport within maize seedlings. The uptake solution contained 0.01 mM atrazine. Otherwise as in Fig. 5.

An increase of the atrazine concentration (0.1 mM) in the bathing solution resulted in an increased uptake and translocation; the apparent pH differences were not significant (Fig. 7).

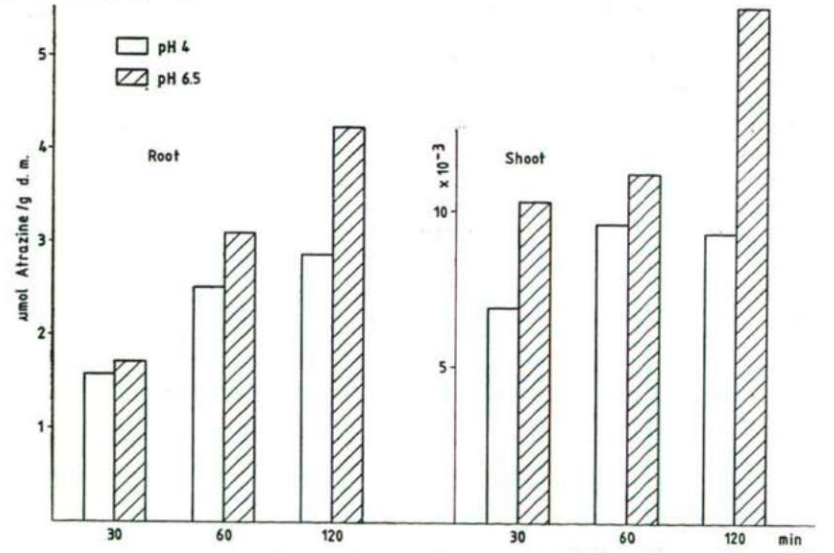


Fig. 7. Effects of pH on uptake of ^{14}C-atrazine and its transport within maize seedlings. The uptake solution contained 0.1 mM atrazine. Otherwise as in Fig. 5.

Discussion

The results obtained with the auxin-type herbicide on the ion uptake by maize roots at different pH values (Figs. 1 and 3) essentially agree with those found for wheat and rice (ZSOLDOS et al., 1978; ZSOLDOS and HAUNOLD, 1982). Since 2,4-D was markedly more strongly absorbed at pH 4 than at 6.5, the inhibition of the ion uptake was to be expected (Figs. 5 and 1). The inhibition of the K uptake increased with time as the 2,4-D concentration in the roots increased, too. Earlier work had shown that active ion uptake by roots was blocked at a 2,4-D concentration of 0.1 mM, a concentration which was likely to be reached within 2 hours of absorption.

Though phenoxy herbicides are readily absorbed by roots at low pH, the translocation to the shoots tends to be restricted (Fig. 5). These results are in full agreement with data from the literature (CRAFTS and YAMAGUCHI, 1960; SCOTT and MORRIS, 1970; ZSOLDOS and HAUNOLD, 1979). The poor translocation of root-applied phenoxy herbicides apparently results from their strong retention by living cells during their passage through the symplast (LOOS, 1975).

As regards atrazine, which is a herbicide of the non-auxin-type, no impeding influence on the ion uptake was observed. The herbicide itself was readily taken up by the roots, though it did not reach the same concentration as 2,4-D (Figs. 5 and 6). Since maize is able to inactivate this herbicide quickly (KEARNEY and KAUFMAN, 1975), the ion uptake proceeded unimpaired; this was the case even when the atrazine concentration in the roots increased by a factor of 20—30 as a result of raising the herbicide concentration in the bathing solution from 0.01 to 0.1 mM. After one hour of uptake the atrazine content of the roots per 1 g d. m. had reached 2—3 μmol, the phosphate content 3—4 μmol and the potassium concent 20 μmol (Table 1). The roots without herbicide treatment took up similar amounts of K and P.

Table 1. Effects of 0.1 mM ^{14}C-atrazine on ion uptake after 1 h (the values given are in μmol/g d. m. in the roots)

	K		*P*		^{14}C-atrazine
	control	herbicide treatment	control	herbicide treatment	—
pH 4	20	21	3.2	4.2	2.5
pH 6.5	22	28	2.6	4.1	3.1

As far as practical agriculture is concerned, the nutrient uptake by maize plants will not be affected even if the atrazine concentration in the soil solution reaches 0.1 mM; furthermore, the pH will not be critical. With 2,4-D, the nutrient uptake is very much reduced in acidic soils if the herbicide in the soil solution reaches a concentration of 0.01 mM; this should be kept in mind when applying the weed killer.

Acknowledgement

The work was carried out under the Austro-Hungarian Scientific and Technical Agreement, Project No. 10100 and 51-1-523. The authors wish to express their thanks to all those organizations which provided help towards the successful performance of the work. Thanks are also due to Mrs. I. SZABÓ and Mr. K. BLOCHBERGER for their excellent assistance in the laboratory work.

References

AMMON, H. U. and HERI, W. J. (1979): Weed control in European maize growing. In Maize Ciba-Geigy Agrochemicals. — Technical Monograph, pp. 46–50.

CRAFTS, A. S. and YAMAGUCHI, S. (1960): Absorption of herbicides by roots. — Am. J. Bot. *47*, 248–255.

HAUNOLD, E. and ZSOLDOS, F. (1976): Der Einfluss von 2,4-D und MCPA auf die Aufnahme und Abgabe von ^{86}Rb(K) und ^{32}P durch Weizenwurzeln. — Die Bodenkultur *27*, 331–338.

JACHETTA, J. J. and RADOSEVICH, S. R. (1981): Enhanced degradation of atrazine by corn (*Zea mays* L.). — Weed Science *29*, 37–44.

KEARNEY, P. C. and KAUFMAN, D. D. (1975): Herbicides. Chemistry, Degradation and Mode of Action. Marcel Dekker Inc. New York and Basel. Vol. *1*.

KENNEDY, D. C. and STEWART, R. A. (1980): The effect of 2,4-dichlorophenoxy-acetic acid on ion uptake by maize roots. — J. Exp. Bot. *31*, 135–150.

LOOS, M. A. (1975): Phenoxyalkanoic acids. In Herbicides. Chemistry, Degradation and Mode of Action. (Eds. P. C. KEARNEY and D. D. KAUFMAN). Marcel Dekker Inc. New York and Basel. Vol. *1*, pp. 1–128.

PETERSON, C. A. and EDGINGTON, L. V. (1976): Entry of pesticides into the plant symplast as measured by their loss from an ambient solution. Pestic. Sci. *7*, 483–491.

PRICE, T. P. and BALKE, N. E. (1982): Characterization of rapid atrazine absorption by excised velvetleaf (*Abutilon theophrasti*) roots. Weed Sci. *30*, 633–639.

SCOTT, P. P. and MORRIS, R. O. (1970): Quantitative distribution and metabolism of auxin herbicides in roots. — Plant Physiol. *62*, 665–669.

ZSOLDOS, F. (1971): Ion uptake by cold injured rice roots. — Plant and Soil *37*, 469–478.

ZSOLDOS, F., KARVALY, B., TÓTH, I. and ERDEI, L. (1978): 2,4-D induced changes in the K uptake of wheat roots at different pH values. — Physiol. Plant. *44*, 395–399.

ZSOLDOS, F. and HAUNOLD, E. (1979): Effect of pH changes on ion and 2,4-D uptake of wheat roots. — Physiol. Plant. *47*, 77–80.

ZSOLDOS, F. and HAUNOLD, E. (1982): Influence of 2,4-D and low pH on potassium, ammonium and nitrate uptake by rice roots. — Physiol. Plant. *54*, 63–68.

Address of the authors:
PROF. DR. E. HAUNOLD
Institute of Agriculture,
Austrian Research Centre,
A-2444 Seibersdorf, Austria
PROF. DR. F. ZSOLDOS
Department of Plant Physiology,
Attila József University,
H-6701 Szeged,
P.O. Box 654, Hungary

Acta Biol. Szeged. 30, pp 27–32 (1984)

STUDIES ON THE TRANSPORT-PHENOMENA WITH EXCISED AND INTACT WHEAT ROOTS

ILDIKÓ TÓTH and F. ZSOLDOS

Attila József University, Department of Plant Physiology, Szeged

(Received June 20, 1983)

Abstract

Authors studied the membrane-bound ATP-ase activity, K^+ uptake and 2,4-D sensitivity of young wheat roots, and compared the transport characteristics of the excised roots and intact plants. The obtained results could be summarized as follows:

1. The 0.01 mM 2,4-D in the uptake solution evidenced greater inhibition of the ATP-ase enzyme functioning in the case of the excised roots than in that of the intact plants. This inhibitory effect was always found to be more expressed in the plants grown in 5×10^{-4} M $CaSO_4$ solution than in those grown in complete nutrient solution.
2. The Viets-effect as well as the effect of the various stress factors was demonstrable both in the case of excised and intact plants, however, in the latter case the K^+ uptake stimulated by Ca^{2+}, and the K^+ content was higher.
3. The differing transport-phenomena experienced between the excised and intact plants could be explained by the joint effect of the various environmental factors and the excision.

Key-words: excised and intact roots, ATP-ase activity, ion uptake .

Introduction

The excised roots have been since long the favoured experimental objects of researchers dealing with ion transport (EPSTEIN, 1972). Nevertheless, it is also known that under certain conditions the ion uptake of the excised roots may vary from that of the intact plants (GLASS, 1978; PARRONDO et al., 1976). It has frequently been experienced in our own experiments that the results obtained with excised roots show greater variation than the results of experiments carried out with whole plants. SACCOMANI et al., (1981) have reported that the uptake of sulphate ion shows greater variation in the case of excised maize roots than in the case of intact plants. The measurements regarding the bulk of the sulphate pool also provide differing results in correlation with the type of plant used in the experiment.

During the course of our experiments the transport characteristics of the excised roots and intact plants were compared from several view-points. The preparable membrane-bound ATPase activity, the changes taking place in the enzyme on the effect of pH stress and 2,4-D were studied. The Ca^{2+}-activated K^+ uptake as well as the pH-dependency of the process was also examined.

Materials and methods

Wheat seedlings were used for the experiments (*Triticum aestivum* L. cv. GK-Szeged). The plants were grown according to the conditions reported on earlier (ZSOLDOS et al., 1978). In accordance with the character of the experiments the nutrient solution was 5×10^{-4} M $CaSO_4$ and modified Hoagland nutrient solution, respectively. For the comparison of the transport-phenomena of the excised roots and intact plants, one part of the plants was placed in the uptake solution series (containing 5 mM KCl, 0,5 mM $CaCl_2$ in the presence and absence of 0,01 mM 2,4-D) in intact condition (shoot and root system together), in the other part only the excised roots were placed in the solution series. The pH of the solutions was checked at the beginning and end of the experiments and the uptake systems were regularly bubbled. After 3 hours the amount of K^+ uptake was measured (ZSOLDOS et al., 1978), the ATP-ase enzyme of the root was prepared and the activity was measured according to data published earlier (ERDEI et al., 1977). The experiments were repeated three times and the present paper comprises the results of a characteristic series.

Results and evaluation

Figure 1 demonstrates the transport ATPase activity of the intact plants. On the upper part of the Figure (A) the data of the roots grown in 5×10^{-4} M $CaSO_4$ are shown, while the lower part (B) demonstrates the results of plants grown in Hoagland nutrient solution. Leaving the wheat roots in uptake solution of pH 4 for a period of 3 hours resulted a higher activity of the prepared ATPase enzyme than in the case of the roots of pH 7. Comparing the plants kept in $CaSO_4$ and nutrient solution, in the latter case the enzyme activity was found to be higher than pH 7. There was a significant difference in the change of activity observed on the effect of 0,01 mM 2,4-D. In the case of the plants grown in $CaSO_4$ an inhibition of 28% was measured at pH 4, while this was 20% in the case of the plants grown in nutrient solution. It was of further interest that at pH 7, 2,4-D did not cause significant changes in the case of $CaSO_4$-grown roots, while the degree of inhibition was found to be 15% in the case of those grown in nutrient solution.

Comparing the results with the data of Figure 2 further interesting differences are gained. The growing of the plants and the setting of the experimental systems was the same as the previous, only in present case excised roots were used. The inhibition of 2,4-D was much more expressed than in the case of the intact plants. The higher ATP-ase activity measured at acidic pH also appeared in these experiments. Regarding the roots kept in $CaSO_4$ (pH 4) in the presence of 0,01 mM 2,4-D, the enzyme activity was lower by more than 40%, and by 30% in the case of the plants kept in nutrient solution. At pH 7 an inhibition over 17% was measured in the case of the $CaSO_4$-grown roots, contrary to the intact plants where these was no significant change. Opposite effect was experienced also in the case of the roots grown in nutrient solution; a stimulation of low degree was observed regarding the excised roots, while an inhibition of more than 15% was obtained in the case of the intact plants.

According to the earlier studies 2,4-D, at acidic pH, significantly inhibited the NH_4^+, NO_3^-, K^+ and phosphate ion uptake of excised wheat roots (ZSOLDOS and HAUNOLD, 1979), as well as the K^+, NH_4^+, and NO_3^- uptake of excised rice roots (ZSOLDOS and HAUNOLD, 1982). Since the active ion uptake takes place demonstrably on the ATP-ases localized in the membrane or with the intervention of ATPase-like proteins (LEONARD and HOTCHKISS, 1976; TÓTH, 1980; ERDEI et al., 1982), the changes in transport could also be concluded from our present investigations.

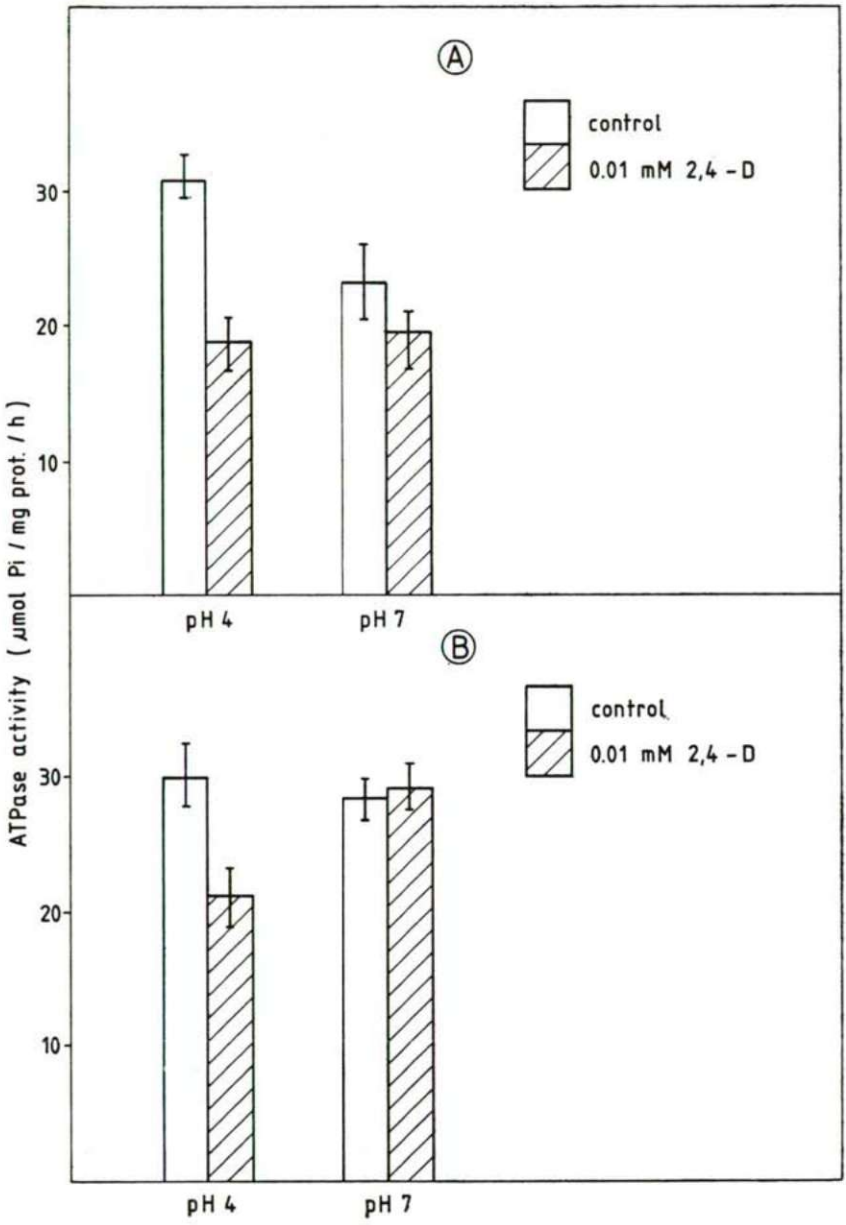


Fig. 1. Membrane-bound ATP-ase activity of 1 week old wheat roots (*Triticum aestivum* L. cv. GK-Szeged) grown in 5×10^{-4} M $CaSO_4$ (A) and modified Hoagland nutrient solution (B), at varying pH and in the presence and absence of 0,01 mM 2,4-D. The uptake solution contained 5 mM K^+ and 0,5 mM Ca^{2+}, the experimental period was 3 hours. Intact plants were used in the experiment.

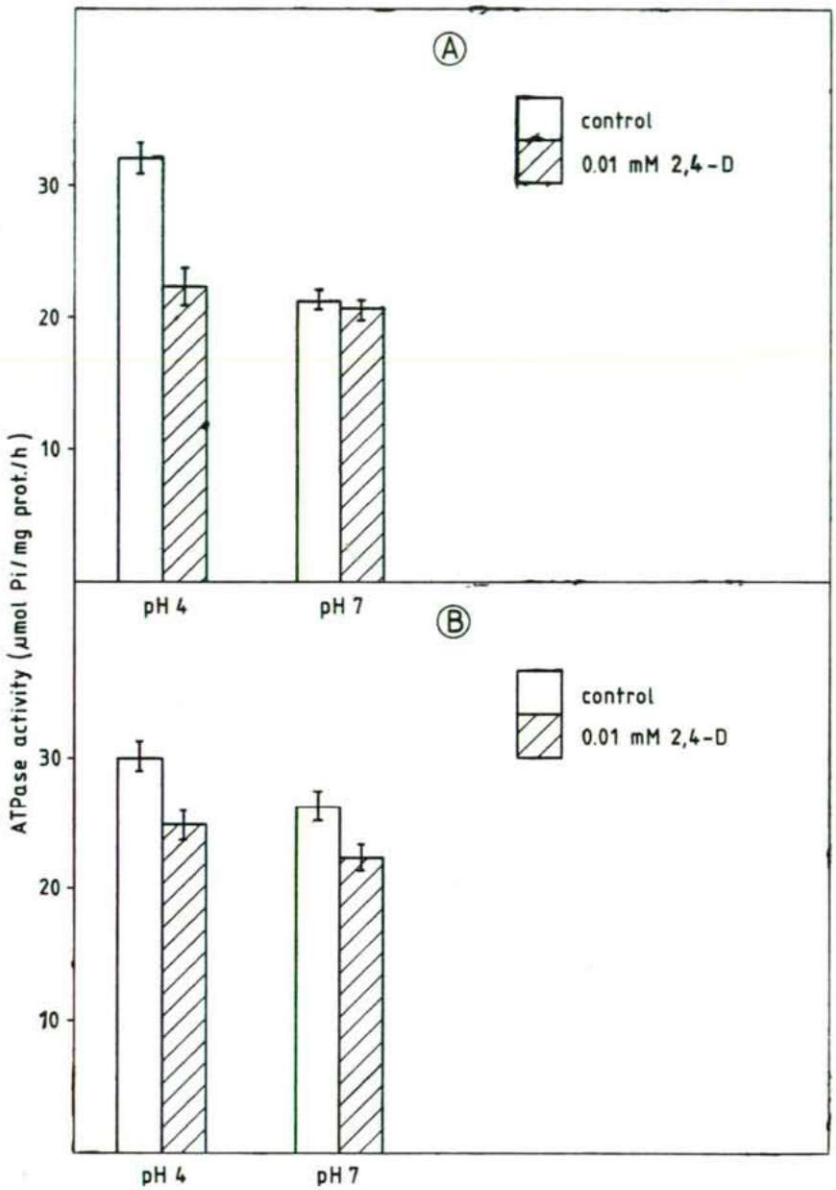


Fig. 2. Membrane-bound ATP-ase activity of 1 week old wheat roots (*Triticum aestivum* L. cv. GK-Szeged) grown in 5×10^{-4} M $CaSO_4$ (A) and modified Hoagland nutrient solution (B), at varying pH and in the presence and absence of 0,01 mM 2,4-D. The uptake solution contained 5 mM K^+ and 0.5 mM Ca^{2+}, the experimental period was 3 hours. Excised roots were used in the experiment.

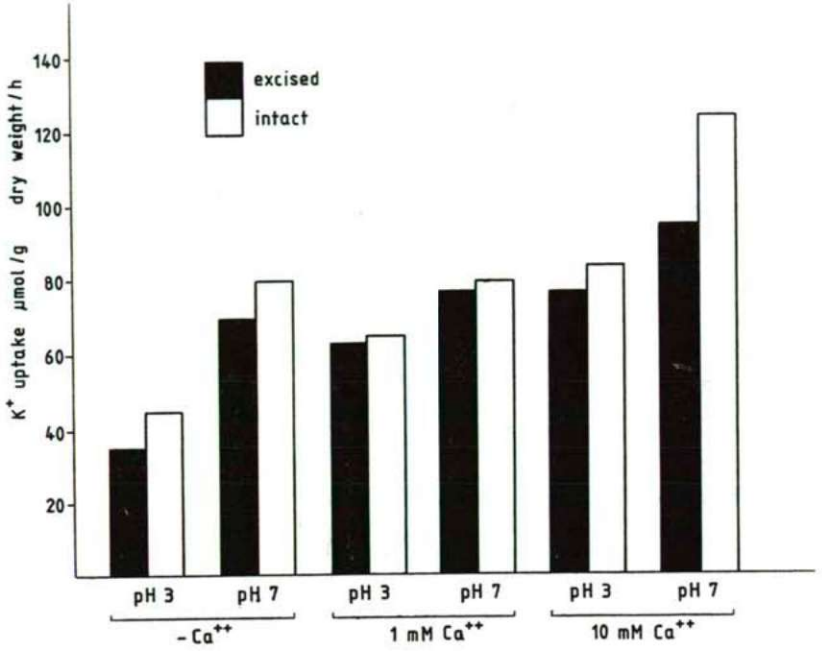


Fig. 3. K^+ uptake of excised and intact wheat roots in the presence and absence of Ca^{2+}, besides varying pH conditions. The uptake solution contained 5 mM K^+ and 0,5 mM Ca^{2+}, the experimental period was 1 hour.

Figure 3 shows the development of the K^+ uptake measured in the presence and absence of Ca^{2+}, in the case of excised roots and intact plants. It could be determined that in the case of the intact plants, the Viets-effect (VIETS, 1944) — that is the K^+ uptake stimulated by Ca^{2+} — was more expressed in every case and in general, the K^+ uptake was higher than in the case of the excised roots.

On the basis of our results it could be concluded that although the excised roots — due to their easy handling — were rather ideal experimental plants for the studies on ion transport, the results obtained are not always in accordance with the happenings taking place in the intact plants. The obtained results show a greatly higher variation and contrary effects may be measured many times. The excision of the root, the separation of the root system from the shooting — the ceasing and modification, resp., of the xylem and phloem transport — is such a stress factor which influences the physiology of the roots; in the present case, the transport characteristics. Since there are plants probably sensitive and less sensitive to stress effects, the question necessitates further studies.

Acknowledgement

Authors express their sincere thanks to MÁRIA TANDARI for her outstanding help in the experiments.

References

EPSTEIN, E. (1972): Mineral Nutrion of Plants: Principles and Perspectives. — John Wiley and Sons, Inc. New York, London, Sydney, Toronto.

ERDEI, L., TÓTH, I. and ZSOLDOS, F. (1977): Potassium absorption by rice at different levels of organization. II. ATP-ase activity in roots, calluses and redifferentiated roots. — Physiol. Plant. *41*, 105–108.

GLASS, A. D. M. (1978): Influence of excision and aging upon K^+ influx into barley roots. — Plant Physiol. *61*, 481–483.

LEONARD, R. T. and HOTCHKISS, C. W. (1976): Cation-stimulated adenosine triphosphatase activity and cation transport in corn roots. — Plant Physiol. *52*, 6–12.

PARRONDO, R. T. and SMITH, R. C. (1976): Effect of removal of the root tip on the development of enhanced Rb^+ absorption by corn roots. — Plant Physiol. *57*, 607–611.

SACCOMANI, M., CACCO, G. and FERRARI, G. (1981): Efficiency of the first steps of sulfate utilization by maize hybrids in relation to their productivity. — Physiol. Plant. *53*, 101–104.

TÓTH, I. and ZSOLDOS, F. (1980): ATP-ase activity and its connection with the active K^+ uptake of different segments of rice and wheat roots. — Acta Biol. Szeged. *26*, 117–121.

VIETS, F. G. (1944): Calcium and other polyvalent cations as accelerators of ion accumulation by excised barley roots. — Plant Physiol. 446–486.

ZSOLDOS, F., KARVALY, B., TÓTH, I. and ERDEI, L. (1978): 2,4-D induced changes in the K^+ uptake of wheat roots at different pH values. — Physiol. Plant. *44*, 395–399.

ZSOLDOS, F. and HAUNOLD, E. (1979): Effects of pH changes on ion and 2,4-D uptake of wheat roots. — Physiol. Plant. *47*, 77–80.

ZSOLDOS, F. and HAUNOLD, E. (1982): Influence of 2,4-D and low pH on potassium, ammonium and nitrate uptake by rice roots. — Physiol. Plant. *54*, 63–68.

Address of the authors:
DR. ILDIKÓ TÓTH
PROF. DR. F. ZSOLDOS
Department of Plant Physiology,
Attila József University
H-6701 Szeged, P.O. Box 654,
Hungary

Acta Biol. Szeged. 30, pp. 33–37 (1984)

THE EFFECT OF PROLINE AND INDOLE ACETIC ACID ON TOTAL AMINO ACID CONTENT OF ALFALFA SHOOTS IN THE CASE OF WATER-DEFICIENCY

G. PÁLFI, ZSÓFIA PÁLFI and ERZSÉBET KÖVES

Department of Plant Physiology, Attila József University, Szeged,
Institute of Plant Physiology, Hungarian Academy of Sciences, Szeged

(Received November 16, 1983)

Abstract

Basal parts of isolated alfalfa shoots were placed in distilled water, and in aqueous solutions of proline, IAA, and ABA; they were standing there in light during 24 hours. Water uptake and transpiration of the shoots standing in ABA and proline solution were lower and quantity of bound water higher than those of the shoots standing in pure water. After the 24 hours of absorption the shoots were laid out for two days (also in light) subjecting them to a sublethal water-deficiency. This stress caused in the shoots standing previously in distilled water a 410% increase of the total amino acid content as compared to the shoots dried immediately after cutting off. An additional 56% increase of the total amino acid content was observed in the shoots which absorbed previously proline and a 15% increase in the shoots which absorbed previously IAA. According to these result proline may have an important role in the watereconomy of the shoots — as IAA and ABA do.

Key-words: ABA, IAA, *Medicago sativa,* Proline waterdeficiency stress.

Introduction

It was already established that when shoots of herbaceous plants are cut off and laid out in light after loosing the greater part of their water content the total free amino acid concentration considerably increases (LEWITT, 1980; PALEG and ASPINALL, 1981). Under the influence of the stress of water loss the proline is the amino acid the concentration of which increases in the highest degree (BATES et al., 1973; TYMMS and GAFF, 1979). DASHEK and ERICKSON (1981) and FELLENBERG (1981) observed that during the first 6—8 hours of the development of waterdeficiency from the active components of the shoots the concentration of IAA-oxydase and of ABA abruptly increases, and at the same time the quantity of IAA considerably decreases. Besides the high ABA concentration considerably increases the synthesis of free proline in the leaves (PALEG and ASPINALL, 1981; QUARRIE and HENSON, 1982; RAJAGOPAL, 1981; TJANKOVA et al., 1982) established that exogenous proline increases the stomaresistency of leaves. Authors investigated how exogenous ABA, IAA, and proline changes water-economy of isolated alfalfa shoots in the case of abuindant water supply. They also investigated the effect of these substances on the free total amino acid content of the shoots during the development of a sublethal deficiency of water in two days.

Materials and methods

Four weeks old shoots of Medicago sativa var. Europe growth under optimal conditions were cut off and weighed in five groups each containing 50 pieces (fresh weight) .The shoots of one group were immediately dried (dry weight, without any manipulation). The shoots of other four groups were put (basal parts below) in aqueous solutions of ABA (4×10^{-5} M), IAA (10^{-4} M), proline (4.3×10^{-3} M) and in pure distilled water. Absorption was performed 24 hours in constant illumination (22 W m^{-2}), temperature was 26 °C and relative humidity 60 per cent. After 24 hours it was determined by weighing how much water was absorbed. Thereafter the shoots were laid out and constantly illuminated for two days causing sublethal water deficiency. Subsequently the shoots were weighed, then split up and dried at 70 °C. Amino acids were determined from the air dry matter with the aid of a "BIOCAL BC 200" automatic analyser. Soluble total protein was extracted from the live-wilted shoots with tris-buffer (pH 7.5) and determined according to Lowry et al. (1951).

Results and discussion

The IAA solution induces epinastic flexoions of alfalfa shoots proving the entry and efficiency. Closing of the stomata was observed in every two hours by microscope. In 6 hours ABA caused a total closing, while proline caused about a 50% decrease of the stomatal opening. Accordingly the agents found entrance into the shoots. Quantity of the absorbed water also shows considerable vitality of the isolated shoots during the 24 hours incubation (Table 1.).

Table 1. Effect of biologically active substances and the proline on water economy and on soluble total protein content of isolated alfalfa shoots in the case of considerable water deficiency. Control: The shoots were dried immediately after cutting off.

Treatment of isolated alfalfa shoots	Data relate to 100 g fresh weight of alfalfa shoots					Total protein as related to the weight after water deficiency mg/g
	water uptake	bound water	evaporated water	after two days of water deficiency		
	in one day			wilting weight	dry weight	
Control	—	—	—	—	19.3	20.4
Water absorption live-wilted	117.2	12.8	104.4	46.5	19.3	19.6
ABA absorption and live-wilted	80.1	15.4	64.7	53.6	19.3	19.5
IAA absorption and and live-wilted	121.6	13.1	108.5	46.7	19.0	20.4
Proline absorption and live-wilted	89.2	17.9	71.3	52.4	19.5	20.7

(Average deviation being below ±5 per cent; n=4).

According to the data in Table 1. the shoots in ABA solution absorbed 31.6% less water than the controls standing in pure water. It is surprising, however, that also absorption of proline caused a considerable (24%) decrease of water absorption;

this was already mentioned by FELLENBERG (1981) and RAJAGOPAL (1981). Order of quantity of the water evaporated by the shoots corresponds to the quantity absorbed. Quantity of bound water is the highest in the presence of proline which shows that proline is effective not only trough the control of the stomatal openings but also through other physiological factors (PALEG and ASPINALL, 1981; TYANKOVA, 1982). According to VAN DE DIJK (1981) in connection with drought-resistance it should be considered that an identical level of „outer water deficiency" produces a lower grade of "inner water deficiency" in varieties better adapted to drought tolerance. Table 2. shows changes in the free amino acid content.

Table 2. Changes in free amino acid content in isolated alfalfa shoots as effected by water, ABA, IAA and proline solutions in the case of considerable water deficiency. Control was dried immediately after cutting off.

Amino acids	Control	Substances absorbed for 24 hours			
		Water	ABA	IAA	Proline
		mg/g dry matter			
Asp	1.50	4.51	4.57	4.52	5.76
Thr	0.59	1.76	1.21	1.34	1.67
Ser	0.95	3.31	3.68	3.57	3.65
Asn	1.66	4.24	3.75	4.28	5.17
Gln	1.30	2.55	2.45	3.54	8.40
Pro	0.35	12.03	17.16	13.29	26.64
Glu	2.08	6.47	6.54	6.41	8.37
Gly	0.58	3.46	3.45	3.83	3.58
Ala	1.13	3.18	3.63	4.15	4.32
Val	0.51	1.79	2.05	3.40	3.16
Cys	0.36	1.17	1.28	1.27	1.55
Met	0.34	1.09	1.15	1.60	1.63
Ile	0.42	2.26	2.28	2.75	2.77
Leu	0.66	2.85	3.14	3.66	3.84
Tyr	0.49	2.15	2.06	2.02	2.73
Phe	0.47	2.03	2.15	2.56	3.18
Trp	0.20	0.92	0.89	1.15	1.16
Lys	0.51	1.52	1.62	1.87	1.93
His	0.53	1.88	1.76	2.33	2.41
Arg	0.40	2.47	2.54	3.31	4.34
Total amino acids	15.03	61.64	67.36	70.85	96.26

(Average deviation being below ± 5 per cent; $n=4$).

According to the data of Table 2. during the development of lethal water deficiency the total amino acid content of the shoots absorbing previously pure water increased 410% as compared to that of the shoots fixed immediately after cutting off. The concentration of proline became thirtyfourfold. The highest differences were produced by the water deficiency. Exogenous proline caused an additional 56% increase of the total amino acids. At the same time IAA caused a 15% while ABA

a 9% additional increase of the total amino acids as compared to the shoots standing in pure water. In the case of ABA increase of the total amino acids is nearly totally attributed to the increase of proline.

Authors conclude that in alfalfa shoots proline induces in the water economy and thereafter during the development of the water deficiency in the total amino acid content similar changes as do IAA and ABA. Moreover, opposite tendencies of the effects of IAA and ABA seem to becombined in the effect of proline. Authors' data prove that proline considerably facilitates tolerance of water deficiency. At the proline-type plants (PÁLFI et al., 1974) we have established that a high proline concentration is advantageous and we are supporting that as follows:

1. The hygroscopic nature of proline and its water-fixing capacity is the highest among all the protein-forming amino acids. Its water-solutibility is standing in the first place, too: at syntheses and transaminations, the most frequently involved glutamic acid is soluble 192-times, and aspartic acid 300-times more poorly in water than proline does. It may therefore be in the tissues of plants in a dissolved, active state, even in the more and more decreasing water.

2. During hydrolysis the free amino acids with 6n HCl, for 24 hours, at 110 °C, under pressure — in the presence of KNO_3 as oxidizer — every protein-forming amino acid was decomposed, except for proline. The proline-stability is extremely high.

3. Proline, during its being formed from glutamic acid, is storing reducing energy coming from photosynthesis and that gets released after the water deficiency being ceased and proline reverting to glutamic acid again. Owing to this redox property, proline has a respiration-influencing role, as well (LEWITT, 1980; PALEG and ASPINALL, 1981).

4. The high concentration of free proline in the tissue and pollen — as compared with the other amino acids — is favourable to growth because it is less toxic, as we have proved that with germinating experiments carried out under sterile conditions and with an oat coleoptil test (PÁLFI et al., 1974, 1981; PINTÉR et al. 1979).

5. In addition, proline is an important component of the proteins of the cell wall during growth and division of the cells. But following its incorporation it is converted to hydroxyproline (DASHEK and ERICKSON, 1981; FELLENBERG, 1981; LEWITT, 1980; PALEG and ASPINALL, 1981; PÁLFI et al., 1974; TYANKOVA et al., 1982).

References

BATES, L. S., WALDREN, R. P. and TEARE, I. D. (1973): Rapid determination of free proline for water-stress studies. — Plant and Soil. *39*, 205–207.

DASHEK, W. V. and ERICKSON, S. S. (1981): Isolation, assay, biosynthesis, metabolism, uptake and translocation of proline in plant cells and tissues. — Bot. Rev. (Bronx) *47*, 349–385.

FELLENBERG, G. (1981): Pflanzenwachstum. Gustav Fischer Verlag, Stuttgart, New York.

LEWITT, J. (1980): Responses of plant to environmental stresses. *II*. 25–76. Acad. Press, New York, San Francisco, London.

LOWRY, O. H., ROSENBROUGH, N. J., FARR, A. L. and RANDALL, R. J. (1951): Protein measurement with the folin phenol reagent. — J. Biol. Chem. *193*, 265–275.

PALEG, G. and ASPINALL, D. (1981): Water stress in plants, 40–183. Acad. Press, New York.

PÁLFI, G., KÖVES, E., BITÓ, M. and SEBESTYÉN, R. (1974): The role of amino acids during water-stress in species accumulating proline.. — Fyton. *32*, 121–127.

PÁLFI, G., PINTÉR, L. and PÁLFI, Zs. (1981): The proline content and fertility of the pollen of inbred maize lines. — Acta Bot. Acad. Sci. Hung. *27*, 179–187.

PINTÉR, L., KÁLMÁN, L. and PÁLFI, G. (1979): Determination of drought resistance in maize (*Zea mays* L.) by proline test. — Maydica. *24*, 155–159.
QUARRIE, S. A. and HENSON, I. E. (1982): Measurement of abscisic acid content of cereal leaves using expressed sap. — Z. Pflanzenphysiol. *108*, 365–373.
RAJAGOPAL, V. (1981): The influence of exogenous proline on the stomatal resistance in *Vicia faba*. — Physiol. Plant. *52*, 292–296.
TYANKOVA, L., TRIFONOV, A. and KUSMANOVA, R. (1982): A spectroscopic (ATR) approach to the behaviour of proline- and sucrose-treated biomembranes towards dehydratation and rewatering. — Biochem. Physiol. Pflanzen. *177*, 509–514.
TYMMS, M. J. and GAFF, D. F. (1979): Proline accumulation during water stress in resurrection plants. — J. Exp. Bot. *30*, 165–168.
VAN DE DIJK, S. J. (1981): Two ecologically distinct subspecies of *Hypochaeris radicata* L. III. Differences in drought resistance. — Plant and Soil. *63*, 149–163.

Address to the authors:
DR. G. PÁLFI and DR. ERZSÉBET KÖVES
Department of Plant Physiology,
Attila József University,
P.O. Box 654; H-6071 Szeged, Hungary
ZSÓFIA PÁLFI
Institute of Plant Physiology
Biological Research Center
Hungarian Academy of Sciences
H-6701 Szeged, P.O. Box 521
Hungary

Acta Biol. Szeged. 30, pp. 39–50 (1984)

ANGABEN ÜBER DIE BIOLOGISCHE UND HEILENDE WIRKUNG DER HÉVIZER TORFMOORSCHLAMM-DERIVATE UND IHRER HUMMUSSTOFFE

G. PÁLFI

Lehrstuhl der Pflanzenphysiologie der Attila József Universität, Szeged

(Eingegangen am 15. 8. 1983)

Zusammenfassung

Wir untersuchten die biologischen Eigenschaften des Hévizer Teiches und den Torfmoor-Schlamm in der Keszthelyer Umgebung und suchten dabei einen Zusammenhang zur Heilwirkung. Dabei stellten wir fest, dass der mit Heilwasser bedeckte Torfmoor-Schlamm einen wesentlich niedrigeren Gehalt an Gesamtstickstoff enthält, als man ihn auf Wiesenmoor findet, der mit Wasser nicht bedeckt ist. Wir zeigten, dass nicht nur der Schlamm des Teiches solch grossmolekulare organische Stoffe, wie die Humussäuren enthält, sondern auch das Thermalwasser in gelöstem Zustand. Die bedeutende biologische Aktivität der Schlamm-Derivate wird nit der Stimulierung des Keimpflanzenwurzel-längenwachstums um 50–100% bewiesen. Das Abwelken von isolierten Weizentrieben in hypertoner Salzlösung gelang uns auf biologischem Weg mit den organischen Stoffen des Hévizer Torfschlammes zu antagonisieren bzw. durch die Hilfe seiner harten aktiven Strukturen. Die baktericide Eigenschaft des Schlammes zeigten wir anhand der Agardiffusionsmethode in zwei verschiedenen Testbakterien.

Die baktericide Wirkung des Hévizer Schlammes wurde auch mit den gewonnenen "sauberen Huminsäure Derivaten" reproduziert. Die aufgezählten biologischen — bzw. baktericiden Wirkungen spielen wahrscheinlich im Verlauf der Thermal-Schlamm-Behandlung eine Rolle in Héviz.

Schlüsselwörter: Torfmoor-Heilschlamm, Gesamt-Stickstoff-Inhalt, Huminsäure, biologische Aktivität, baktericide Wirkung, die Mikrostrukturen des Schlammes.

Einführung

Der Naturteich in Héviz ist auf Grund seiner Fläche (3,6 ha) und seiner Tiefe (36,8 m) der grösste in Europa. Dieses schwefelhaltige, schwach radioaktive Thermalwasser enthält viele Arten wertvoller Mineralsalze. Die vielen gelösten anorganischen chemischen Zusammensetzungen und die biologische Wirkung im Thermalwasser und im Schlamm kennen wir schon (BREZNAY, 1970; STRECKER, 1970; ZSIRAI und STRECKER, 1970 usw.) Ähnliche Salzzusammensetzungen wie in Héviz kann man auch in anderen Schlammen Ungarns finden. Wodurch können wir nun dem Schlamm des Hévizer Teiches eine besonders intensive biologische Aktivität zuschreiben?

Der Hévizer Schlamm und das Thermalwasser unterscheiden sich dadurch von anderen Thermalgewässern, weil am tiefsten Teil des Teiches wasserreiche Quellen heraussprudeln, die den Teich ständig in Bewegung halten. Das pausenlos strömende Thermalwasser kann mit dem sich auf dem Boden befindenen Torf-Moor-Schlamm in Wechselwirkung treten und kann dadurch anorganische und grossmolekulare organische Stoffe auslösen, darunter auch Huminsäuren (GUPTA et al., 1982).

Nach der völligen Zermalmung der harten Schlamm-probe-Strukturen bzw. nach ihrer Homogenisierung der gewonnenen wässrigen Derivate, liessen wir winzige Kerne keimen. (PÁLFI und SRECKER 1975; PÁLFI et al., 1976). Wir zeigten, dass die Derivate des Thermalmoores auch biologisch aktive Stoffe enthalten.

Mit aerober, steriler Agardiffusionsmethode stellten wir fest, dass das Schlammderivat die Vermehrung der reinen Züchtung des *E. coli* — Testbakteriums hemmt (Bakteriostatische Wirkung).

Das Ziel für unsere Untersuchungen ist, klarzustellen, ob der sich auf dem Teichboden befindende Torfmoor-Schlamm des Thermalwassers grossmolekulare organische Stoffe in gelöstem Zustand enthält.

Mit der Keimung der Kerne in sterilen Umgebungen haben wir die biologischen Aktivitäten der Wasserderivate der homogenisierten Thermalschlamme weiterverfolgt bzw. die Stimulierung des Wurzelwachstums beobachtet.

Wir untersuchten, ob die schädliche wasserentziehende Wirkung der künstlich-zusammengesetzten hypertonischen, anorganischen Salzlösungen an den Bohnentrieben von den Schlammderivaten antagonisiert wird.

Wir fertigten aus dem Hévizer Torfmoor-Schlamm reines Huminsäurederivat an; später untersuchten wir auch die baktericiden Eigenschaften und die biologische Aktivität der von anorganischen Salzen gereinigten Humaten.

Wir untersuchten die biologischen harten, feinen Strukturen des Torfmoor-Schlammes sowie die Morphologie und Plazierung der aus Grossmolekulen bestehenden agglutinierten dunklen Humate. Wir bemühten uns, zu klären, welches die Ursache in der Umgebung sei, die das Teichwasser verschmutzt bzw. sein chemisches Gleichgewicht am meisten beeinträchtigt.

Nach FEKETE et al., (1967) hat der Torf in der Landwirtschaft den Vorteil, dass er eine grosse Adsorptionsfähigkeit, biologische Aktivität und eine baktericide Wirkung besitzt. In unseren Untersuchungen geben wir weitere Angaben über die Keszthelyer Umgebung bzw. über den Hévizer Torf bekannt. SZALAI und SZILÁGYI (1968), BELÁK et al., (1969) und SZALAI et al., (1974) stellten fest, dass die Humussäuren des Torfs durch die starke Adsorption von Spurenelementen Mangelkrankheiten an Pflanzen hervorrufen. BELÁK et al., (1970), SZALAI et al., (1970, 1975) bewiesen in ihren Experimenten, dass man den genannten Mikroelementmangel durch Düngersprühung beseitigen kann. SIPOS et al., (1974) stellte fest, das die aus dem Keszthelyer Flächenmoor-Torf gewonnenen Huminsäure Proben praktisch hydrofyl sind, die Huminsäuren des Lignit und der Braunkohle sind aber bis zu einem bestimmten Grad lipofyl. (Unsere Torfproben aus der Umgebung von Keszthely sind also hydrofyl.) Nach Lakatos et al. (1974) sind die aus der Keszthelyer Umgebung der Flächenmoor-Torfe vorgestellten Huminsäuren solche Biopolimere, die als biologisch aktive Verbindungen in der Landwirtschaft eine ausserordentliche Bedeutung innehaben.

Material und Methode

Wir entnahmen Torf-Schlamm-Proben aus dem Hévizer Teich, aus seinem abfliessenden Kanal und von den sich in der Umgebung des Kanals befindenden Auen aus 10 und 30 cm Tiefe. Um den Schlamm haltbar zu machen, wird er aus dem Abflusskanal des Teiches entnommen und im Sommer auf betonierter Unterlage zum Trocknen ausgelegt, un später in fein gemalener torf aufgehoben zu werden.

Die Zusammensetzungen der löslichen Stoffe im Teichwasser haben wir so bestimmt, indem wir die Schlamm-Proben vier Stunden lang mit destilliertem Wasser schüttelten und danach wurde die nach der Setzung und Dekantierung gewonnene Flüssigkeit geschleudert. Danach verdünsteten wir auf 100 °C 100 ml saubere Flüssigkeit (in viermaliger Wiederholung) und massen den Trockensubstanzgehalt. Das so gewonnene Gesamt Trockensubstrat wurde 4 Stunden lang auf 500 °C geglüht. Der Rest ergab nach der Abkühlung und Messung die Gesamtmenge der anorganischen Salze, den durch das Glühen eingetretenen. Gewichtsverlust, den gesamtorganischen Stoff.

Die Menge der gelösten grossmolekularen Stoffe wurde dadurch bestimmt, indem wir das anorganische kleine molekularmassige Salz des Teiches vorher durch Dialyse entfernten.

Da der Schlamm eine grossflächige, netzartige oder schwammartige Beschaffenheit besitzt, hält er viele Arten von Verbindungen durch seine harte Struktur fest. Die anorganischen und organischen Stoffe des Schlammes und seine nassen Derivate haben wir so hergestellt, dass wir den Thermalschlamm auf 70 °C bis zur Gewichtsbeständigkeit austrockneten und danach homogenisierten wir jeweils 10 g in 30 ml destilliertem Wasser in Reibschalen. Durch das danach folgende Schleudern gewannen wir saubere, farblose Derivate, die aber anorganische und organische Stoffe in löslichem Zustand enthielten. Im Verlauf der Kernkeimung befeuchteten wir die Filterpapiere der Petrischale mit sterilisierten Schlammderivaten. Die so hergestellten feuchten Derivate verwendeten wir für die biologische Aktivitätsmessung und zum Nachweis der baktericiden Wirkung des feuchten Schlammes.

Das mit Hefeauszügen ergänzte harte Fleischagar stellten wir nach FERENCZY und ZSOLT (1971) zusammen. Die Agardiffusionsmethode der baktericiden Wirkung haben wir ebenfalls nach den vorhergenannten Autoren durchgeführt. Die sauberen Züchtungen der Bakterien haben uns auch FERENCZY und ZSOLT zur Verfügung gestellt.

Wir fertigten eine 2% Gesamtsalz beinhaltende Lösung an, die aus Ka, Na, Ca, Magnesiumchlorid und Nitrat bestand. Wenn wir die abgeschnittenen Bohnentriebe in diese Lösung gaben, liessen sie nach 24 Stunden die Blätter hängen — also sie zeigten eine starke Welkung. Die Humate des Torfschlammes deckten wir mit 0,125 n NaOH auf nach LAKATOS et al. (1974). Nach Filtrierung und Schleudern erhielten wir eine etwas dunkle, braun-schwarze Lösung. Den pH-Wert des Huminsäurederivats stellten wir mit 1 n Salzsäure auf 7 ein, das entstandene NaCl und die sonstigen Salze entfernten wir mit Dialyse. Bei diesem Vorgang haben wir die Arbeiten von DRAGUNOV und KABLOVA (1974) in Betracht gezogen.

Wir untersuchten die harte Konstruktion des Schlammes nach Wasserverdünnung in Abstrichen. Die mikroskopische Vergrösserung war 500-fach, die Vergrösserung der Bildaufnahmen dagegen 4-fach wie auch die Abbildungen mit annähernd 2000-facher Vergrösserung gezeigt werden.

Für die Bewertung der Wasserverschmutzung des Thermalteiches wuschen wir von einer dm^2 Human-Hautoberfläche mit 20 ml 30%-igem Äthanol die freien Aminosäuren ab und analysierten die gewonnenen Lösungen (Einzelheiten dazu bei PÁLFI et al., 1974, 1976).

Den Nachweis des löslichen Gesamtproteins erbrachten wir nach LOWRY et al., (1951) die Lichtabsorbierungsintensität der Lösungen massen wir mit dem Spektrofotometer.

Die Menge der Wiederholungen der Analysen beträgt 3 oder 4. Wenn vom Durchschnittsergebnis bei einigen Analysen die Fehlermultiplikation 5% überschritt, wiederholten wir den ganzen Prozess.

Ergebnisse und ihre Auswertung

Die Messungen nach Kjeldahl-Zerstörung und Stickstoff Kolorimetrie der Schlammproben aus dem Thermalwasser auf Gesamt-Stickstoff-Inhalt (organischer und anorganischer Stickstoff) zeigten in keinem Fall 1% der Gesamt-Trockensubstanzmenge. Zu gleicher Zeit lag aber bei den benachbarten nicht durch Wasser bedeckten (und nicht ausgewaschenen) Schichten der Gesamt-N-Inhalt der Torfböden zwischen 2 und 3%. Wir stellten fest, dass die organischen Verbindungen des Stickstoff-enthaltenden Schlames nicht zu den freien Aminosäuren, der Peptide, der Eiweise, der Nukleinsäuren und nicht zu ihren Basen gehören und keine Karbamid- oder Ammonium-Verbindungen sind.

Was für besonders wirkende organische Säuren mögen sie sein? Sind sie auch im gelösten Zustand des Teichwassers nachweisbar oder sind sie nur im Torfmoor-Schlamm enthalten?

Nachdem wir das Teichwasser mit Torfmoor-Schlamm vier Stunden lang durchschüttelten, gewannen wir nach Setzen, Dekantierung, Filtrieren und Zentrifugieren eine saubere farblose Fülssigkeit, die wir bis zur Trockenheit verdunsteten und dann massen wir eine 560 mg/L Gesamttrocken-Substanz. Mit der weiteren Analyse der getrockneten Substanz und nach dem Verglühen der organischen Stoffe, stellten wir fest, dass beinahe die Hälfte der gelösten Stoffe des Teichwassers (268 mg/L) aus Grossmolekülen bestehende stickstoffarme; hauptsächlich zu Huminsäuren gehörende organische Stoffe sind. Die schon erwähnten organischen Verbindungen sind nicht nur im aufgewühlten Schlamm enthalten, sondern von ihnen wird eine bedeutende Menge des fliessenden Wassers aufgelöst. Das Teichwasser wurde bis jetzt noch nicht nach seinen gelösten und organischen Stoffen untersucht, auch ihre biologische Aktivität noch nicht.

Mit der Keimung der winzigen Kerne (PÁLFI und STRECKER, 1975) zeigten wir schon, dass bei einigen Pflanzenarten die Schlammderivate das Wurzelwachstum bedeutend stimulieren. Mit grossen Kernen ist es aber noch nicht gelungen, so eine Aktivität nachzuweisen. Besonders diejenigen, die zur Gruppe der Hülsenfrüchte gehören und über eine grosse Menge Nährstoff-Reserven verfügen; wie z. B. : Bohnen, Erbsen und Linsen ergaben negative Ergebnisse. Diese Kerne beinhalten während ihrer Keimung in bedeutender Menge synthetisierende, aktivierende und mobilisierende Stoffe, die das endogene Wachstum stimulieren. Deshalb ist die äussere Dosierung der aktiven Stoffe weniger wirkend.

Unsere Versuche bezüglich auf die begünstigende Wirkung des Wurzelwachstums bei Linsenkernen mit grossen Reservenährstoffmengen waren auch erfolgreich, wenn die Schlammderivate optimal konzentriert und der pH-Wert richtig eingestellt war.

Die Abbildungen 1 und 2 auf der Tafel 1 zeigen die Kerne der oberen Reihe, die im Leitungswasser keimten (kontrolle); die in der unteren Reihe dagegen stammen aus den Hévizer Schlammderivaten. Die Wurzeln der unteren Reihe wuchsen im Falle der Linsen 50% länger gegenüber der Kontrolle (Abb. 1). Die Wachstum-Stimulierung (Tafel 1, Abb. 2) bei dem kleinkernigen Radieschen (*Raphanus sativus* L.) um 100%. Der Torfmoor-Schlamm enthält bedeutende biologisch-aktive Stoffe.

Tafel 1, Abb. 1 und 2 — Die stimulierende Wirkung der Torf-Moor-Schlamm-Derivate beim Wurzelwachstum. Die Pflanzen der oberen Reihe keimten im Leitungswasser (Kontrollen), die der unteren in den feuchten Derivaten des Torf-Moor-Schlammes steriles Medium). Die Schlamm-Derivate stimulierten das Wurzel-Längenwachstum.

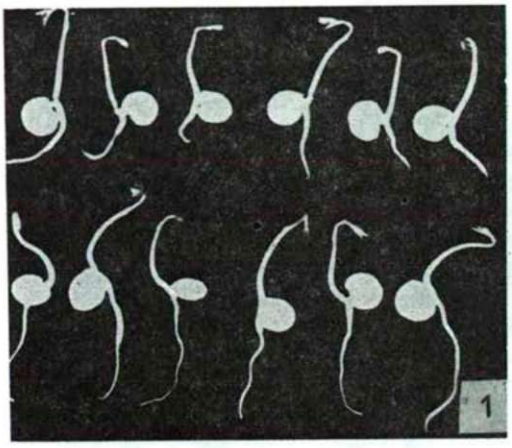
1. Linsenpflanzen (*Lens culinaris* L.); der Wurzelwachstum beträgt 50%.
2. Radieschenpflanzen (*Raphanus sativus* L.) — die Stimulierung des Wurzel-Längenwachstums beträgt 100% (die Bildvergrösserung ist ca. fönffach).

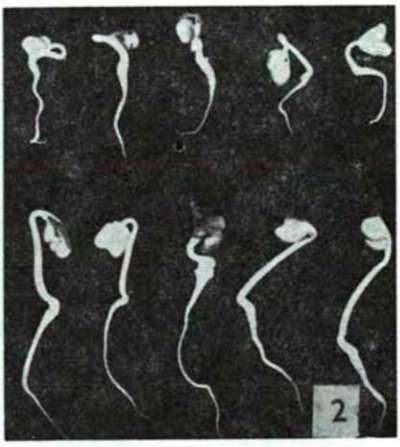
Abb. 3, Die Verringerung der wasserentziehenden Wirkung der hypertonischen Salzlösungen mit Torf-Moor-Schlamm. Der erste Kolben auf der linken Seite enthält sauberes Leitungswasser, das ist die Kontrolle. In die darauffolgenden 6 Kolben rechts gaben wir hypertone (wasserentziehend) Salzlösung und je Kolben 6, 4, 3, 2 und 1 g getrockneten und zu Pulver gemalenen Torf-Moor-Schlamm. Der äussere Kolben rechts enthält nur Salzlösung.

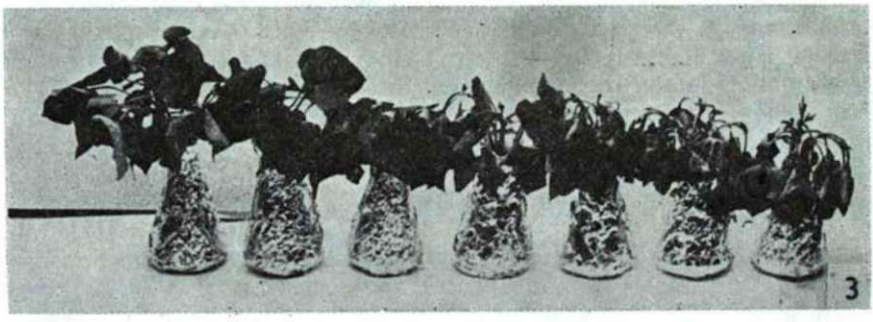
Abb. 4. und 5. Der Beweis der baktericiden Wirkung des Torf-Moor-Schlammes mit Agar-diffusion. Wir dosierten in die drei oberen Höhlen, die auf beiden Bildern zu sehen sind, Streptomycinlösungen (25, 50 und 100 mg/L), in den unteren drei Höhlen sind die „feuchten Derivate" des sterilen Torf-Moor-Schlammes. Die ausgebildeten Bakterien-Kolonien (aus den Höhlen ausdiffundiert) töteten nicht nur die Antibiotikum-Lösungen, sondern auch die Schlammderivate.

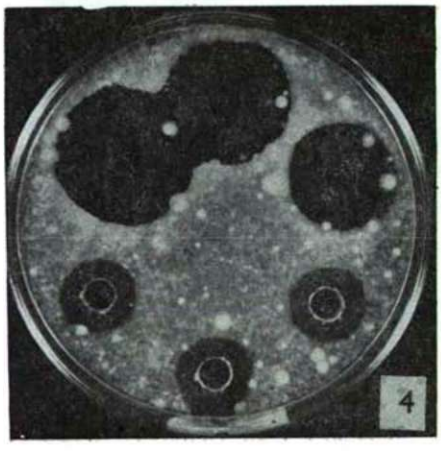
Abb. 4 = *Bacillus cereus var. mycoides;*

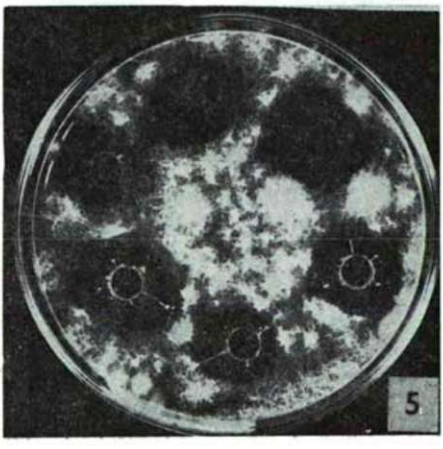
Abb. 5 = *Staphylococcus aureus.*


1


2


3


4


5

Diese Stoffe, eindringend durch das pflanzliche Hautgewebe, beschleunigen den Steffwechsel, wie sich das im Laufe der Wurzelwachstum-Anspornung äussert.

Für den Wurzelwachstum der Keimpflanzen sichert die Stimulierung in der Landwirtschaft einen bedeutenden Vorteil. Die längeren und besser entwickelten Keimpflanzen dringen nämlich schneller in die Tiefe des Bodens und erreichen so die feuchten Schichten eher; sie sichern der Pflanze innerhalb kurzer Zeit Wasser- und Nährstoff-Versorgung, wodurch im Endergebnis das Verderben der Keimpflanzen prozentual reduziert werden kann. Wahrscheinlich ist dies der Vorteil der Torfanwendung beim Ziehen der Setzlinge in der Gartenwirtschaft.

Die beobachtete stimulierende Wirkung der Torf-Schlamm-Derivate bei der Keimung von Kernen könnte auch eine Rolle in der Balneologie bzw. in der humanen Heilbehandlung spielen.

Wenn wir die Triebe der weichstengligen Pflanzen in die erhöhten osmotischen Druck zeigenden Salzlösungen stellen, verlieren die Blätter ihre Turgor und welken innerhalb von 24 Stunden wegen des starken Wasserverlustes ab.

Im Laufe unserer Versuche gossen wir in 6 Erlenmeyer Kolben je 100 ml 2% ige Gesamtsalz enthaltende, physiologoisch gesehen, hypertonische Lösung; als Kontrolle aber wandten wir destilliertes Wasser an. Auf 5 Gefässe Salzlösung dosierten wir fein gemalenen luftgetrockneten Schlammstaub in stufenweise verminderten Mengen (6, 4, 3, 2 und 1 g). In einem Gefäss blieb also nur reine Salzlösung die die hypertonische Kontrolle war. In jeden Kolben gaben wir die gleiche Anzahl isolierten Bohnentriebe, dann inkubierten wir die Gefässe bei 25 °C mit 60% Feuchtigkeit der Luft und 4000 lux Beleuchtung während 24 Stunden. Auf der Abb. 3 der Tafel 1 war im ersten Gefäss reines Wasser (Kontrolle), das keinen Wasserentzug bzw. kein Welken der Bohnenblätter auslöste. Das zweite Gefäss von links enthält 2%-iges Gesamtsalz, aber die dazugegebenen 6 g Torfstaub antagonisierte die schädliche Wirkung der hypertonischen Lösung, deshalb verwelken hier auch die Bohnenblätter nicht. In den folgenden vier Gefässen enthält die Salzlösung den Torfstaub nur noch in reduzierter Menge und die Welkung der Bohnenblätter und ihr Herunterhängen nimmt dementsprechend zu. Der letzte Kolben rechts aussen enthält reine Salzlösung und die Verwelkung dieser Triebe ist offensichtlich die stärkste.

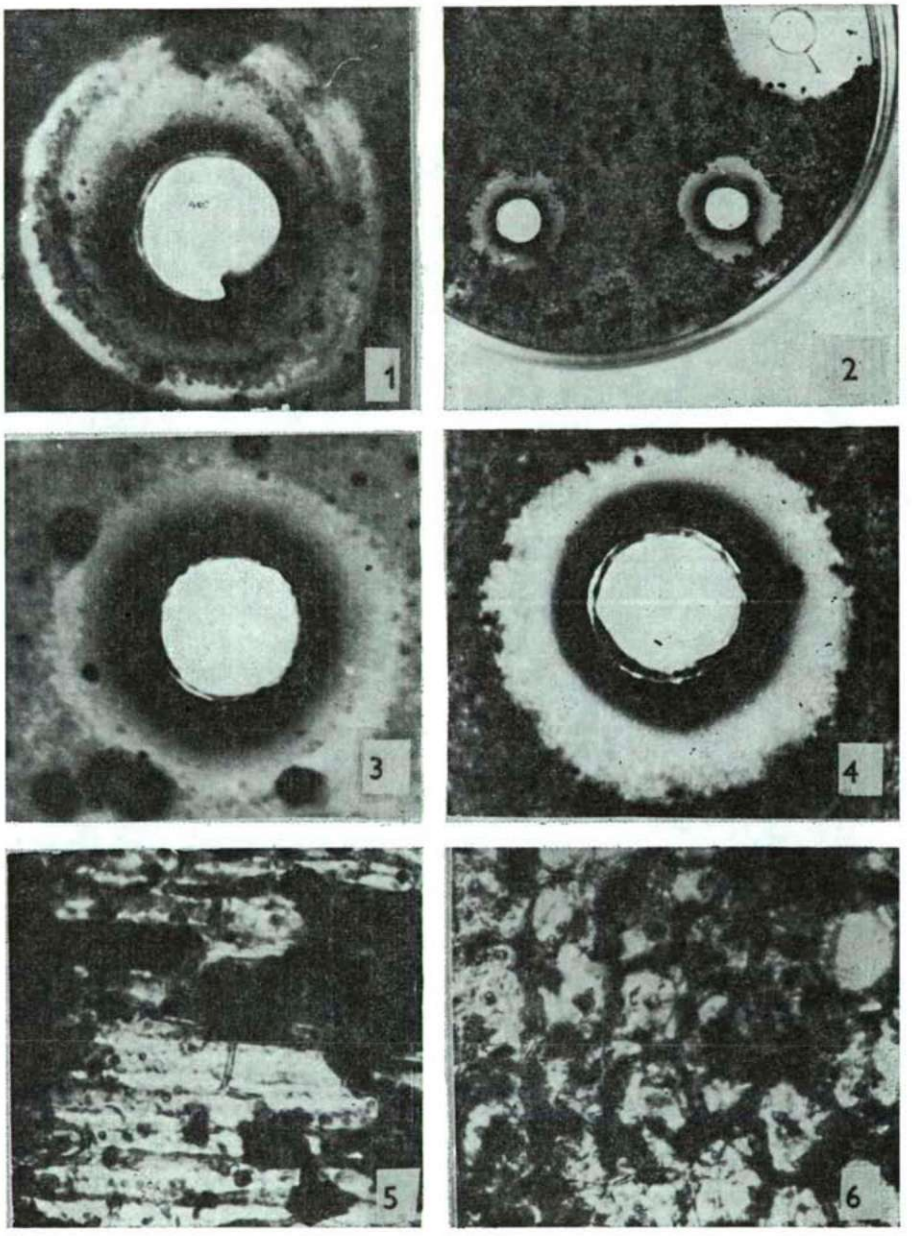
Wie ist diese Erscheinung zu erklären? Es ist wahrscheinlich, dass der Hévizer Torfmoor-Schlamm neben biologischaktiven Stoffen eine grosse Menge solcher grossflächiger organischer und anorganischer harter Strukturen und Makromoleküle

Tafel 2, Abb. 1, 2, 3 und 4. Die Agardiffusionszonen der aus Torf-Moor-Schlamm hergestellten reinen Huminsäure-Derivaten. Abb. 2. *Staphylococcus aureus*. Aus der oberen Höhle ist die Streptomycin-Lösung (Kontorlle) ausdiffundiert, ihre abgetötete Zone (weisgewordene Fläche) ist ausgebreitet. Aus den sich darunter links befindenen 2 Höhlen wurden auch die dunkelfarbigen Huminsäuren ausdiffundiert, diese bracthen jedoch nur kleinere Abtötungszone zustande.

Abb. 4. Die Abtötungszone der Huminsäure in vierfacher Vergrösserung *(Staphylococcus aureus)*; der äussere farblose Kreisbogen ist die Zone der Kleinmolekularen Huminsäure, der innere schwarze Kreisbogen ist die Zone der grossmolekulargewichtigen Humine.

Abb. 1. und 3. Die abgetöteten Zonen der Huminsäuren sind vierfach vergrössert, das Testbakterium ist bei beiden der *Bacillus cereus var. mycoides*.

Abb. 5. und 6. Ursprünglicher pflanzlicher Stoff, netzartige Zellwandgerippe mit eingefangenen Huminpartikeln. Abb. 5. Das Zellwandgerippe ist kaum humifiziert. Abb. 6. Das Zellwandgerippe ist mittelmässig humifiziert.



enthält, wie z. B.: Huminsäuren, die die wasserentziehende Wirkung der hypertonen, anorganischen Salzlösungen antagonisieren und damit auf die Pflanzen eine günstige physiologische Wirkung ausüben. Es ist anzunehmen, dass der Wirkungsmechanismus der Staubarten (des Torfs und Lignits), die zur Verbesserung des Salz-Alkalibodens

angewandt wurden, auch ähnlich sind. Auf jeden Fall ist diese Wirkung der Torfe — egal mit welchem Mechanismus es sich verwirklicht — ein solch biologischer Vorteil, der auch im Verlauf der balneologischen bzw. der Heilbehandlung eine Bedeutung hat.

Anhand der schon früher untersuchten mikrobiologischen Eigenschaften des Schlammes stellten wir fest, dass der Schlamm nur in aussergewöhnlich minimaler Menge Bakterien enthält (PÁLFI und STRECKER, 1975; PÁLFI et al., 1976). Die Wechselhaftigkeit der Bakterienarten ist ganz arm und ihre Zahl ist auch sehr niederig. Aber warum ist das so? Zwei Hauptursachen kennen wir schon:

1. - der Stickstoffmangel
2. - der Schlamm hemmt die Vermehrung der Bakterien (Bakteriostatische Wirkung). Diese Eigenschaften stellten wir schon bei den reinen Züchtungen des *E. coli* fest (PÁLFI et al., 1976).

Um was für Verbindungstypen mag es sich bei diesen baktericiden organischen Stoffen handeln? Die Entstehung, Entwicklung und die Struktur des Schlammes in Betracht ziehend, dachten wir in erster Linie an die Huminsäuren. Aus diesem Grunde versuchten wir, die baktericide Wirkung mit den reinen Huminsäure-Derivaten des Torfmoor-Schlammes zu reproduzieren.

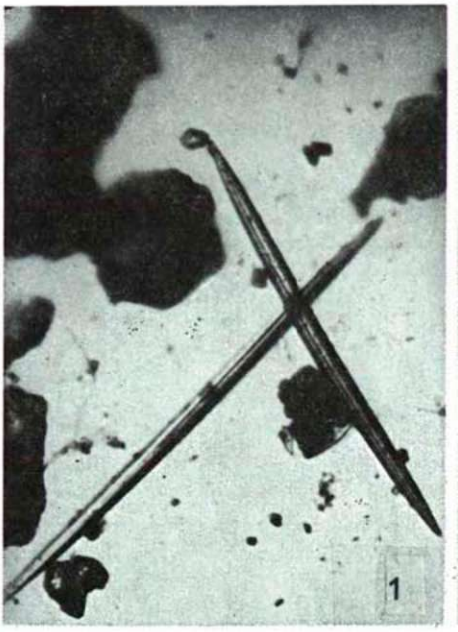
Um die Vergleichbarkeit zu erreichen, wandten wir die international anerkannten abziehenden — und reiningenden Methoden der Huminsäuren an (DRAGUNOV und KABLOVA, 1974; LAKATOS et al., 1974; FORTUN und POLO, 1982; RITCHIE und POSNER 1982; ROLETTO et al., 1982).

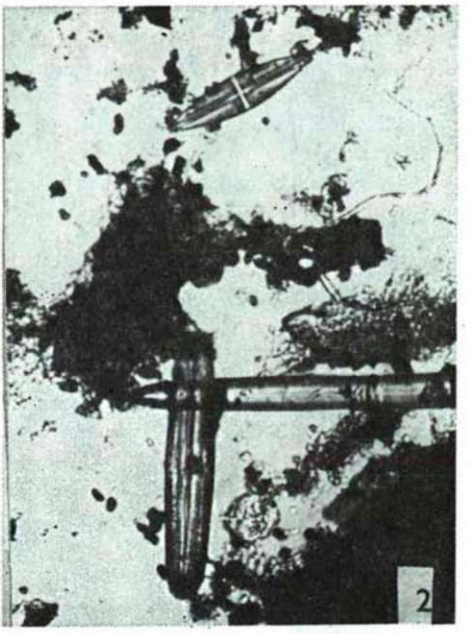
Wir untersuchten die baktericide Eigenschaft der aus dem Torfmoor-Schlamm gewonnenen dunkelfarbigen Huminsäure-Derivate an Bacillus cereus var. mycoides und an Staphylococcus aureus-Test-bakterien. Die Huminsäuren brachten nach 8 tägiger Inkubation bedeutend grosse, kreisförmige Vernichtungszonen zustande, wodurch wir auch die baktericide Eigenschaft nachweisen konnten. (an der Abb. 2 der Tafel 2 ist die Konzentration des Streptomycins 100 mg/L darunter nebeneinander sind Huminsäure-Derivate. Das Testbakterium ist hier *Staphylococcus aureus*).

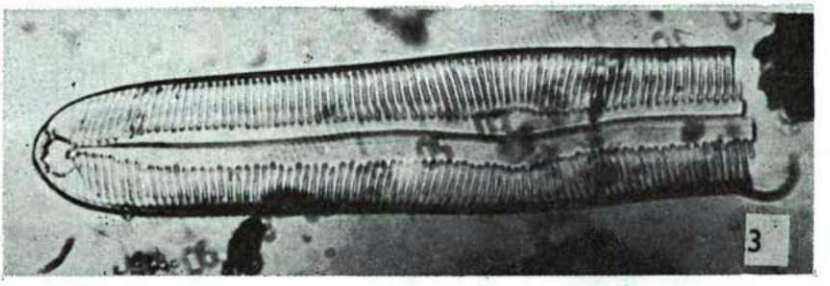
Bei den Agardiffusionskreisen der Tafel 2 ist zu sehen, dass auch die allergrössten Molekül-Massen (20—50-Tausend) aufzweigenden schwarzen Huminsäuren aus den Höhlen des Ernährungsagars ausgewandert sind, aber von den Rändern der Höhle ungefähr nur halb so weit gelangt sind, wie die farblosen kleinmolekularmassigen Huminsäuren (Tafel 2, Abb. 1, 2, 3 und 4).

Die mittelmässig mol-massigen braunen und grauen Huminsäuren (mol-Masse - - 10–20 000) befinden sich in der Mitte der Vernichtungszone (Auf dem Foto sind diese auch schwarz zu sehen). Die allerkleinsten mol-massigen (3–10 000) farblosen wasserlöslichen Huminsäuren diffundierten die Höhlen am antferntesten (grosse

Tafel 3, Abb. 1. Die biologischen, harten strukturen des Torf-Moor-Schlammes.
Die Gerippe der Kowamoose (Diatome). Die Gerippe zweier zur selben Art gehörenden abgestorbenen Diatome. Daneben sind grosse schwarze Huminsäure-Partikel zu sehen. Die an Stickstoff armen Humin-Partikel könnten ursprüngliche Torfmoose sein.
Abb. 2. Diatome-Gerippe, die zu drei verschiedenen Arten gehören, die schwarze Partikel mit Humus bedeckt und die netzartige Konstruktion aufzeigen.
Abb. 3. Diatome-Gerippe-Teil, rauhe Einschnitte und schwarze Humin-Partikel-Teile an der Oberfläche.
Abb. 4. Das Zellwandgerippe ist schon völlig humifiziert, die Zellwand-Netze sind mit schwarzen Humin-Partikeln völlig ausgefüllt.







weisse Kreise). Auf der Abb. 1 der Tafel 2 sind Bakterienkolonien in den äusseren breiten Vernichtungszonen der Diffusion zu sehen, die gegenüber der farblosen Huminsäuren resistent sind.

Auf der Abb. 3 der Tafel 2 an den Rändern der weissen Zone sind auch 3–4 Bakterienkolonien (auf dem Bild schwarz) die gegenüber den farblosen Huminsäuren resistent sind. Auf der Abb. 4 der Tafel 1 sind in der Antibiotikum-Vernichtungszone streptomycin-resistente kleine weisse Kolonien zu finden.

Zusammenfassend können wir feststellen, dass die reinen Huminsäuren des Hévizer Thermalschlammes über solche baktericide Wirkung verfügten, die bei der Schlammbehandlung bzw. bei sonstigen balneologischen Heilwirkungen eine bedeutende Rolle spielen. Können wir aber die wurzelwachstumstimulierenden biologisch-aktiven Stoffe, die bei der Kernkeimung eine Rolle gespielt haben auch zu den Komponenten der Huminsäuren einstufen?

FRENYÓ (1974) neutralisierte aus heimischer Kohle gewonnene Huminsäuren und nach dem Studium entsprechender Verdünnungen hat er Keimungsversuche mit Reis angefangen. Der Autor wies an den Wurzeln der Keimpflanzen die bedeutende wachstumstimulierende Wirkung der Huminsäuren nach. So bestand auch für uns die Hoffnung, dass wir mit den Huminsäuren positive Ergebnisse erreichen werden.

Wir keimten Senf-, Radieschen-, Luzern- und Linsen-Kerne in Hunderter-Serien (bei viermaliger Wiederholung) in Lösungen der unterschiedlich verdünnten sterilen Huminsäure. Die Kontrolle war steriles Leitungswasser.

Die Keimversuche wiederholten wir mehrmals, jedoch der Keimungsprozentsatz im Trieb- oder Wurzelwachstum ergab keinen Unterschied. Der bioaktive Stoff des Torf-Moor-Schlammes befindet sich entweder nicht unter den Huminsäuren oder ist irgend eine andere natürliche Verbindung. Es kann vorkommen, dass dieser Stoff auf die Wirkung des alkalischen Entzugs verdarb oder aber im Derivat die organischen Salze oder andersartige Aktivatoren fehlten (infolge der Dialyse).

Im weiteren entnahmen wir aus der 30 cm Schicht des Teichschlammes Proben, da die Durchschnittstiefe bei der Abtragung des Schlammes diesen Wert besitzt. Wir untersuchten mit dem Mikroskop die harten Strukturen der feinen Schlamm-Konstruktion. Die mikroskopischen Aufnahmen 1, 2 und 3 auf der Tafel 3 veranschaulichen die abgestorbenen harten Gerippe des Kowamooses.

Auf der Abb. 1 der 3. Tafel sind zwei zu der gleichen Art gehörende Diatome zu sehen, daneben Partikel, die von humifizierten, schwarzen Torf-Moos stammen.

Die Abb. 2 auf der Tafel 3 zeigt drei Diatome-Gerippe, die zu drei verschiedenen Arten gehören. Die Zeichnung des Zellengerippes auf der 3. Abb. der Tafel 3 beweist das, dass die ganze Oberfläche der Diatome reibeisenmässig gespalten ist. Diese "scharfen" Konstruktionen machen 2–3% des harten Stoffes beim gesetzten Schlamm aus.

Unter die wirksamen Strukturen können wir die zellwandingen Gerippe der abgestorbenen pflanzlichen Gewebe einreichen (Tafel 2: Abb. 5, 6 und Tafel 3: Abb. 4). Diese Zellwandgerippe zeigen die beginnende Humifikation, aber stellenweise kann man auch die ursprüngliche Zellwand sehen (Tafel 2, Abb. 5). Die Humifikation des Zellwandnetzes kann auch mittelmässig sein (Tafel 2, Abb. 6), die im Fall die Zellwände überall mit Humin bedeckt, jedoch das Netz teilweise noch leer ist. Das Endstadium ist die völlige Humifikation des Zellwandgerippes, wobei nicht nur die Zellwände, sondern auch die Zellhöhlen schwarzes Humin ausfüllt (Tafel 3, Abb 4). Diese abgestorbenen Zellwandgerippe sind die strukturellen Träger der Huminsäuren.

Wir müssen bemerken, dass die chemische Zusammensetzung der Huminsäuren noch unbekannt ist. Um dies aufzudecken, wandte SCHNITZER (1976) das Gaskromatografie-Massenspektrometer-Computer-System an, Dannenberg und ULLACH (1982) führten schon interessante Isolierungsversuche durch, um den strukturellen Aufbau der Humusstoffe zu Klären.

Schliesslich müssen wir in Betracht ziehen, dass die löslichen, in bedeutender Menge vorhandenen organischen Stoffe im Hévizer Thermalwasser nicht als „Umweltverschmutzung" angesehen werden können, weil diese organischen Verbindungen wasserlösliche Huminsäuren sind und keinen Stickstoff in bedeutender Menge enthalten.

Literatur

BELÁK, S., GYŐRI, D., SÁMSONI, Z., SZALAY, S., SZILÁGYI, M. und TÓTH, A. (1969): Mikroelem felvételének tanulmányozása a keszthelyi rétlápon (Die Untersuchung der Aufnahme der Mikroelemente im Keszthelyier Wiesenmoor). — Agrokémia és Talajtan. *18*, 263–288.

BELÁK, S., GYŐRI, D., SÁMSONI, Z., SZALAY, S., SZILÁGYI, M. und TÓTH, A. (1970): A mikroelemek felvételének tanulmányozása a keszthelyi rétlápon. II. Sudáni cirokfű és zab (Die Untersuchung der aufnahme der Mikroelemente im Keszthelyier Wiesenmoor. II. *Sorghum halepense* (L) *var. sudanense* und *Hafer*). — Agrokémia és Talajtan. *19*, 27–38.

BREZNAY, G. (1970): Az iszapkezelésekről (Über die Schlammbehandlung) 193–208. In: ZSIRAI, K. (1970). Második Hévízi Orvosi Archivum. Zalaegerszeg.

DANNEBERG, O. H. und ULLAH, S. M. (1982): Chromatographysche Unterscheidung von Huminstoffen und Nichthuminstoffen aus Schwarzerdehumus. — Z. Pflanzenernähr. Bodenkunde, *145*, 526–538.

DRAGUNOV, S. und KABLOVA, N. G. (1974): Biochimitscheskoje issledovanije torfjannüch guminovüch kislot. — Potschvovedenije. *1*, 52–57.

FEKETE, Z., HARGITAI, L. und ZSOLDOS, L. (1967): Talajtan és Agrokémia (Bodenlehre und Agrochemie). Mezőgazdasági Kiadó. Budapest.

FORTUN, C. und POLO, A. (1982): Efecto de algunos tipos de compuestos humicos sobre el crecimiento de las raices *Zea mays*. — Agrochimica. Pisa. *26*, 44–54.

FRENYO, V. (1974): Káliumhumát és nitrohuminsav együttes hatása rizs csíragyökerének a növekedésére (Die gemeinsame Wirkung des Kaliumhumats und der Nitrohuminsäure auf das Wachstum der Reiswurzelkeimung.) — Botanikai közlemények. *61*, 263–266.

GUPTA, S. K., NÄ NI, H. und SCHINDLER, P. W. (1982): Kolloidchemische Grundlagen des Ton-Humus-Komplexes: Einfluss von pH, Eisen und unterschiedlich eisenhaltigem Kaolinit auf die Löslichkeit von Huminsäure. — Schweiz. Landw. Forsch. Bern. *21*, 67–74.

LAKATOS, B., FRAU T. MEISEL and MÁNDY, GY. (1974): Biopolimer-fém komplex rendszerek. I. Kísérletek nagytisztaságú tőzeg humuszanyagok és fémkomplexeik előállítàsàra. (Biopolimer-Metall Komplex-Systeme. I. Versuche zur Herstellung von grossreinheitigen Humusstoffen und ihren Metallkomplexen), — Agrokémia és Talajtan. *23*, 505–522.

LOWRY, O. H., ROSENBROUGH, N. J., FARR, A. L. und RANDALL, R. J. (1951): Protein measurement with the folin-phenol reagent. — J. Biol. Chem. 193, 256–275.

PÁLFI, G., ERZSÉBET KÖVES, BITÓ, M. und RITA SEBESTYÉN (1974): The role of amino acides during water stress in species accumulating proline. Fyton. *32*, 121–127.

PÁLFI, G. und STRECKER, O. (1975): Hévíz felfedezése. Mikroszkóp alatt a gyógyhatás. (Die Entdeckung von Héviz. Die Heilwirkung unter dem Mikroskop). — Delta. (Hung.) *12*, 17–20.

PÁLFI, G., STRECKER, O. und GUBICZA, A. (1976): Biological activity, bactericidal effect and structure of a medicinal peat-bog mud (at Héviz). — Acta Biol. Szeged. *22*, 57–71.

RITCHIE, G. S. P. und POSNER, A. M. (1982): The effect of pH and metal binding on the transport properties of humic acid. — J. Soil. Sci. Oxford. *33*, 233–248.

ROLETTO, E., BARBERIS, R. und ZELANO, V. (1982): Gel filtration and absorption spectroscopic investigations on humic substances from organic fertilizers. — Plant and Soil. *66*, 383–390.

SCHNITZER, M. (1976): The chemistry of humic substances. Environmental biogeochemistry. — Ann. Arbor Sci. Publishers Inc. *1*, 89–107.

SIPOS, S., KEDVES, É., DÉKÁNY, I., DEÉR, A. FRAU T. MEISEL und LAKATOS, B. (1974): Biopolimer-fém komplex rendszerek. II. Humuszanyagok és fémekkel alkotott rendszereik fizikai tulajdonságai (Biopolimer-Metall Komplex-Systeme. II. Die physikalischen Eigenschaften der mit Humusstoffen und Metall hergestellten Systeme). — Agrokémia és Talajtan. *23*, 313–334.

STRECKER, O. (1970): A fürdőkúrák és a balneológiai kutatás problémái. In: ZSIRAI, K. (1970): Második Hévízi Orvosi Archivum. Zalaegerszeg.

SZALAY, S. und SZILÁGYI, M. (1968): Nyomtápelemek szorpciója a tőzeghumuszsavakon és jelentősége a gyakorlati mezőgazdaságban (Die sorption der Spuren-Ernährungselemente in den Torf-Humussäuren und ihre Bedeutung in der Praktischen Landwirtschaft). — Agrártudományi Közlemények. *27*, 109–117.

SZALAY, S., SZILÁGYI, M. und SÁMSONI, Z. (1970): Mikroelem hiányjelenségek az Enying környéki lápterületen (Mangelerscheinungen der Mikroelemente im Moorgebiet um Enying). — Agrokémia és Talajtan. *19*, 1–12.

SZALAY, S., SÁMSONI, Z. und SZILÁGYI, M. (1974): Magyarországi tőzegtalajok kationszorpciós és mikroelem vizsgálata (Die Untersuchung von ungarischen Torfböden auf Kationssorption und Mikroelemente). — Növénytermelés. *23*, 327–334.

SZALAY, S., SÁMSONI, Z. und SZILÁGYI, M. (1975): Magyarországi tőzeges talajok növényeinek mikroelem hiányjelenségeiről (Über die Mikroelementmangelscheinungen bei Pflanzen auf ungarischen Torfböden). — Növénytermelés. *24*, 35–45.

ZSIRAI, K. und STRECKER, O. (1970): Az iszapképződés biológiai háttere (Der biologische Hintergrund der Schlammbildung). — A Második Hévízi Orvosi Archivum. Zalaegerszeg.

Anschrift des Verfassers:
DR. G. PÁLFI
Lehrstuhl der Pflanzenphysiologie der
Attila József Universität
Postafach 654
H-6701, SZEGED, Ungarn

Acta Biol. Szeged. 30, pp. 51–59 (1984)

EFFECT OF IDENTICAL AND ALTERNATING LIGHT-DARK PERIODS ON THE GROWTH, DRY-MATTER ACCUMULATION AND CARBOHYDRATE CONTENT OF MAIZE LEAVES

I. MARÓTI and KATALIN MARGÓCZI

Attila József University, Department of Botany, Szeged

(Received November 30, 1983)

Abstract

The growth of Pioneer 3839 hybrid maize leaves, the changes in the amount of dry-matter and soluble sugar and starch levels of the leaves were studied for 37 days at identical light-dark periods (LDP) of 16–8 hours, 30–15 min. and 15–7.5 min.; as well as on plants grown in changed LDP-s following the first 22 days of growth. The daily period of light was 16 hours, the light intensity was 32 W/m², and the temperature was 20±2 °C.

It could be determined that there was slight increase in the dry-matter mass of the plants growing for 37 days at identical LDP-s on the effect of the 30–15 min. LD-treatment, and a decrease was observable on the effect of the 15–7.5 min. LDP, compared to the 16–8 hours control.

Among the maize plants grown in alternating LDP-s in every case the dry-weight of ones grown in shorter LDP-s (30–15 min. 15–7.5 min.) for the first 22 days, and than were exposed to 16–8 h LDP for 16 days, — was higher.

The short periodical illumination accelerates the development and longitudinal growth of the young leaves in general. The changes in the LDP-s do not have any effect on the growth rate of the half-developed leaves.

In the developed leaves grown in 30–15 and 15–7.5 min. LDP-s for 37 days the decrese of the soluble sugar content is considerable, and the starch-content shows a decrease of slighter degree. In the 30–15 min. LDP the soluble carbohydrate level of the young leaves (6th, 7th) only slightly varies; the starch amount does not change at all.

From the changed LDP-s applied after 22 days of growth the shorter ones decrease, while the longer ones increase the amount of soluble sugar and starch.

Key-words: light-dark period, dry-weight, soluble sugar, starch, growing, maize.

Introduction

The alternating short light-dark periods (LPD) frequently decrease the relative efficiency of photosynthesis; respectively, it comes close to, but only rarely surpasses the values measured in continuous light (SAGER and GIGER, 1980). This effect is particularly striking in the case of algae, in respect to plants of higher order it appears less unambigously.

From the latest results, on the basis of the studies carries out by NAYLOR and GILES (1982) on bean plants, the 5–5 min. and 1–1 min. LDP-s decreased the dry-weight and leaf surface, and increased the quota of the root. These also decreased the amount and size of starch granules in chloroplasts, and furthermore, had significant on the pigment formation.

There are still relatively few data, regarding the effect of the varying LDP-s on the carbohydrate metabolism. It seems that our knowledge concerning the regu-

lation of the biosynthesis and decomposition of strach is not sufficient enough yet for explaining acceptably the starch level-decreasing effect of the short light-dark periods (PREISS, 1982).

According to the studies of KAISER and BASSHAM (1979) — following a single short, 4 min. dark period the CO_2 fixation and the starch synthesis continues in a slightly decreased degree, although without lag phase.

According to our studies so far (MARÓTI and PATAKY, 1982; MARÓTI and MIHALIK, 1982; TAKÁCS and MARÓTI, 1983; MARÓTI and TAKÁCS, 1983), besides continuous light intensity and identical daily light amount the effect of alternating illumination is determined by the length of the light-dark periods and by the genotype of the maize. On the basis of the changes in dry matter production the P523 maize type is capable of accomodating to the 30–15 and 15–7.5 min. LDP-s the best, while this ability is the least in the case of the P165 maize type.

In the case of the P523 maize, compared to the long-day (16–8 hours) illumination, the 30–15 min. LDP accelerates the development of the leaves increases the leaf surface and the dry weight per unit surface (MARÓTI and MIHALIK, 1982).

In respect to the plants studied so far, the uniform unfavourable effect of the 15–7.5 min. LDP was mainly manifested in the decrease of specific leaf-dry weight, the relative increase of root-weight, and in the significant decrease of chlorophylls and carotenoids (MARÓTI, 1982).

Compared to the parent lines, the P3901 hybrid maize made the best use of the light in every LDP, and its considerable mass of dry matter was accompanied by the high soluble sugar concent of the leaves (MARÓTI and MIHALIK, 1982).

The certain maize types are adaptable to the short LDP depending on their genotype. It is also the characteristic effect of the 30–15 min. LDP that the soluble sugar and starch amount found in the unit dry matter of the leaf decreases depending on the genotype. However the 15–7.5 min. LDP is uniformly and expressedly unfavourable for the accumulation of carbohydrates (MARÓTI and MIHALIK, 1982). The question is, how the soluble sugar and starch change in the leaves of maize adapted to varing LDP-s, and then exposed to a new, shorter or longer LDP? Those leaves are of particular interest from this viewpoint, which grew partly (half time) in the earlier, partly in the new LDP; and respectively, those leaves, which were formed entirely in the new LDP, i. e., the observable initiative leaves appeared after the transfer.

Answering the above question, this paper reports on and discusses the changes in dry matter, soluble sugar and starch levels of Pioneer 3839 type hybrid maize, developing on the effect of identical and alternating LDP-s.

Materials and Methods

Pioneer 3839 hybrid maize (*Zea mays* L.) were grown in phytotron (HORVÁTH, 1972) for the experiments. The temperature was 20 ± 2 °C, the humidity of the air was 50–70%.

The plants were sown in the mixture of sand-perlite in the ratio of 1:1, the nutriment was ensured with modified Hoagland nutrient solution, and the moisture-content corresponding to 80% of water capacity was maintained daily by tap-water with watering according to weight. Two series of experiments were carried out at varying time-points.

F-29 light tubes were applied for illumination (wave-lengths 400–700 nm). The light flux density was 32 W/m². The total illumination of 16 hours daily was continuous in the first climate chamber,

lasting from 8 a.m. to 12 p.m. (16–8 hours light-dark period), in the second chamber alternating periods of 30 min. light and 15 min. dark were applied: 30–15 min. LDP; and in the third, 15 min. light and 7.5 min. dark periods alternated: 15–7.5 min. LDP. The daily total illumination was 16 hours in every chamber.

After growing for 22 days the plants were divided into 3 equal parts in all three climate chambers: 12 plants remained in the original place, the rest of the plants were placed into new LDP-s; 12 into one, 12 into the other. 3–3 plants were selected from each variant and these were followed by daily measuring of the growth in length of their 4th and 5th leaves, counted from the bottom.

The plants were processed at the age of 37 days. The fresh weights of the 12–12 plants per treatment were measured according to organs. This processing was always carried out in the morning hours. The dry weight was measured after drying at 70 °C.

The carbohydrate content was determined from the 4th and from the youngest 6th, 7th... leaves. The dry matter of the appropriate leaf from 7 plants per treatment was combined and cut into entirely small pieces with scissors. From this, 50 mg were ground with some water with the help of quartzsand.

The soluble carbohydrates were extracted with 10 ml hot water, then following centrifugation and discharging, extraction was performed again with newer 10 ml hot water by shaking for 15 min. The two supernatants were blended, filled up to 100 ml, from which the determinations were accomplished.

After extracting the soluble sugars a quantity of 10 ml 35% perchloride acid was poured onto the centrifugal sediment. Following 1 hour of shaking this was centrifuged, then after pouring off the supernatant, a newer amount of 10 ml 35% perchloride acid was added and after 1 hour's shaking the samples were left till the following day. Next day the mixture was centrifuged and the supernatant was combined with the first perchloride acidic fraction. After appropriate dilution, the starch content was determined.

The determination of the soluble sugars and the hydrolysed starch was performed using the method of DUBOIS et al., (1956): 0.5 ml sample + 0.5 ml 5% phenol + 2 ml concentrated sulphuric acid. After 20 minutes the cooled coloured solution was measured by photometry at 485 nm. The calibration curve was prepared with D-glucose.

In the case of each sample the carbohydrate content was determined twice from 50 mg dry matter independently of each other, and the measurings were repeated when they were necessary.

Results

A) The effect of identical and alternating short LDP-s on the accumulation of the dry-matter and percental quota of dryweight of organs in 3839 maize.

It can be seen from the measurings of the dry-weight related to one plant (Table 1) that the mass of dry-matter of the plants grown in same LDP for 37 days was slightly increased by the 30–15 min. LD-treatment and decreased by the 15–7.5 min. LDP compared to the 16–8 hours control. The uniform effect of the short periodical illumination (30–15 and 15–7.5 min) was that it stimulated the development and increase in dry-weight of the young leaves (Figs. 2, 3), and decreased the amount of dry-weight in the developed leaves (Fig. 1).

The 30–15 min. LDP being the same throughout the experiment firstly increased the dry-matter percental quota of the roots (Table 1).

In the case of alternating LDP-s, at the timepoint of transfer to new LDP, the 4th leaf was cc. half developed (the leaf was at about half-time between appearance and complete development), the 5th leaf just appeared and the 6th, 7th leaves appeared in the new LDP during the 16 days.

From the maize grown in alternating LDP-s in every case the dry weight of those which grew in shorter LDP (30–15 and 15–7.5 min) for the first 22 days, and then were transfered to 16–8 hours LD periods for 15 days was higher (Table 1).

A smaller degree of increase in dry-matter compared to the continuous 16–8 hours control was observable even when the plants were placed from longer (16–8 hours and 30–15 min.) LD periods after 22 days to shorter (15–7.5 min) ones.

In the alternating LD periods the accumulation in dry-matter was generally in relationship with the percental increase of the root and leaf sheath.

Table 1. The effect of LDP-s being the same throughout the experiments and changed after 22 days of growing, resp., on the dry-weight (mg/plant) and quota of organs of 37 days old Pioner 3839 maize.

In the 16-8 hours, 30-15 min. and 15-7.5 min. LDP-s the 36-36 identical plants were grown for 22 days, then divided into three according to treatment : 12 plants remained in the originai place, and the rest of the 12-12 plants were grown further in other two LDP-s.

The daily amount of light (512 Wh/m²) was the same in each variant. The plants were processed at the age of 37 days. The data are the averages of the 12-12 plants from the two experimental series.

Light-dark period		Dry-weight (mg/plant)							
of the first 22 days	on the further 15 days	root		leaf sheath + stem		leaf		total	
		mg	%	mg	%	mg	%	mg	%
16-8 hours	16–8 hours	383	39	167	18	415	43	965	100
	30–15 min	383	39	175	18	412	43	970	100
	15–7.5 min	450	40	279	25	388	35	1117	100
30–15 min	16–8 hours	538	45	183	15	379	40	1200	100
	30–15 min	421	43	142	14	418	42	981	100
	15–7.5 min	517	45	159	14	461	41	1137	100
15–7.5 min	16–8 hours	513	43	204	17	478	40	1194	100
	30–15 min	374	39	150	16	426	45	950	100
	15–7.5 min	367	40	150	16	406	44	923	100

The dry-weight of the 4th leaf was strongly decreased by the transfer to shorter LDP, however, that of the same leaf of plants placed from shorter LD period to longer one increased to such extent that it even surpassed the data gained for plants grown throughout in 16–8 hours LDP (Fig. 1). That is, the leaf which obtained advantage in development in shorter LDP, grew for longer period when transfered to longer LDP, therefore its dry-weight became higher than that of the leaf placed with disadvantage in development from longer LDP to shorter LDP. This effect was particularly striking in the case of combinung the 16–8 hours and 15–7.5 min. LDP-s (Fig. 1), but the same effect could be recognized in slighter form in the case of the alternated 16–8 hours and 30–15 min. LDP-s, and even in a less distinct form on the occasion of changing the 30–15 min. and 15–7,5 min. LDP-s, resp. (Fig. 1).

The dry-weight of the young 6th, 7th leaves developing in new LDP after 22 days was regulated by two effects. On the one hand, the short periodical illumination stimulated the development of the young leaves; on the other hand, the maize placed from pre-

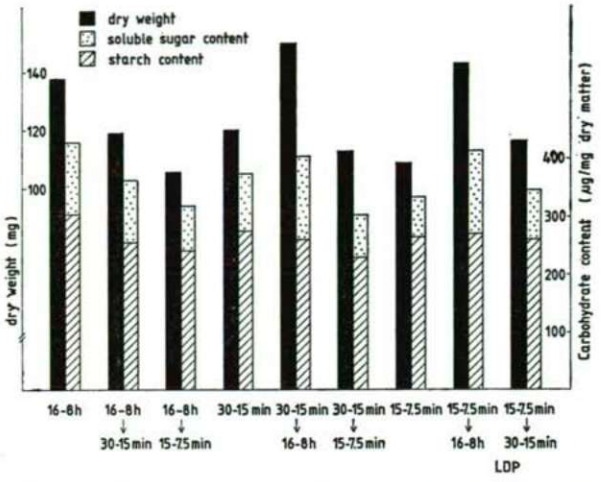


Fig. 1. The carbohydrate content and dryweight of the 4th leaf on the effect of identical and alternating LDP-s.
Experimental conditions: see Table 1.
At the time of transfer to the new LDP the 4th leaf was about half-developed.
The determination of the carbohydrate content was accomplished from the combined and homogenized dry-matter of the 4th leaves from 7 plants per treatment, measuring 50 mg twice.
The data are the averages of 2 experimental series.

vious short LDP to longer LDP produced more dry-matter than those which grew throughout in either short or long LDP. This is why the dry weight of the young 6th, 7th leaves was found to be the least in the case of maize grown entirely in 16–8 hours LDP, from every experimental varient (Fig. 2).

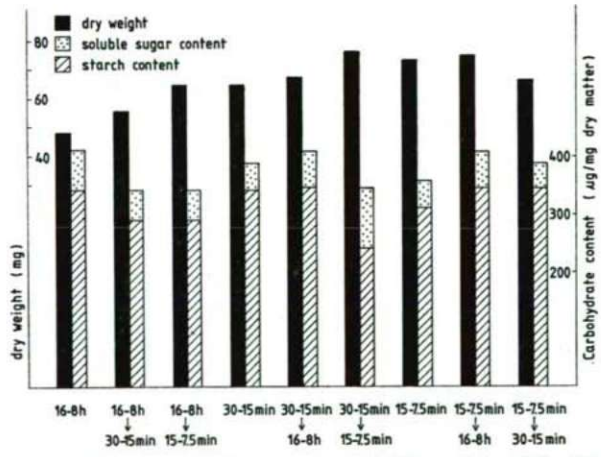


Fig. 2. The development of the carbohydrate content and dry-weight of the 6th, 7th leaves on the effect of identical and alternating LDP-s. For experimental conditions and studying methods see Table 1 and Fig. 1.
The 6th leaf had not appeared yet at the time of transfer to new LDP.

B) The effect of identical and alternating LDP-s on the longitudinal growth of the 4th and 5th leaves of the maize .

The length of the 4th and 5th leaves was measured daily after placing the plants into new LDP (22 days). The end of the 3rd leaf sheath was taken as the lower source of the leaf length. At the beginning of the measurings the 4th leaf was in half-time of development and the 5th leaf had just appeared. On one part, it could be observed from the measurings that the development of the leaves was accelerated by the short light-dark periods and the leaves reached complete development earlier. On the other part it was observable that the effect of the various LDP-s greatly depended on the age and state of development of the leaves.

The new LDP hardly showed effect on the growth of the half-developed 4th leaf, and it grew at the rate characteristic to the original LDP even after transfer (Fig. 3).

At the time of change in LDP, the 5th leaf had only started to grow, and it rapidly became adapted to the growth rate characteristic to the new LDP. It can also well be seen that at the beginning the plant grew more rapidly in the 15–7.5 min LDP (Fig. 3).

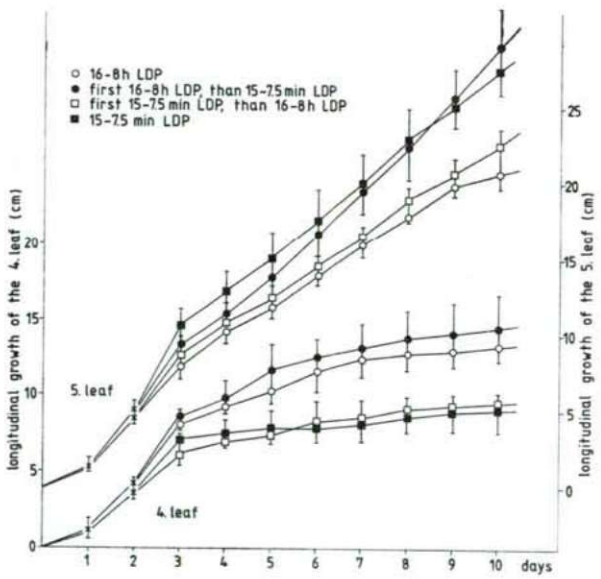


Fig. 3. The effect of identical and alternating LDP-s on the longitudinal growth of the 4th and 5th leaves.
The measurings were commenced when the plants were 22 days old, at the time of their placing into new LDP. The source of the length-measurings was all throughout started from the lower edge of the 3rd leaf's lamella since at this time-point the lingule and auricle of the undeveloped 4th leaf had not been formed yet, the leaf was in cc. half-time state of growth, and the 5th leaf had only just appeared.
The measurings were taken for a period of 10 days from 3–3 plants daily.

C) The effect of identical and alternating LDP-s on the carbohydrate content of the developed (4th) and developing (6th, 7th) leaves.

The shorter light-dark period decreased the level of soluble sugar and starch in the case of the studied leaves; that of the soluble sugar at higher degree, and that of starch at lower degree (Figs. 1, 2). Related to dry-weight unit, the soluble carbohydrate content of the 4th leaf showed 33% decrease in the 30–15 min. LDP compared to the 16–8 hours LDP, and a decrease of 53% was experienced in the 15–7.5 min LDP (Fig. 1). The change in starch level was slighter: of 10–20%.

The carbohydrate level of the developing leaves (6th, 7th) showed lower decrease in the case of 30–15 min. and 15–7.5 min. LDP. In the 30—15 min. LDP there was no, or only slight change in the level of starch (Fig. 2).

In the developing leaves in every case the starch level was found to be higher, and that of soluble sugar was lower, than in the developed leaves. It is presumable that the plant "ensures" higher starch level for the intensive growth of the young leaves nevertheless, the energy-consumption implying intensive growth keeps the soluble sugars at low level, i. e. there is no "stagnant" stock of soluble sugar.

This assertion is in agreement with the results of KAISER and BASSHAM (1979), as well as PREISS (1982), according to which the starch found in the leaf can easily be mobilized and is controlled by the effective regulation of the metabolic processes related to the various energy productions and consumptions.

The decrease in starch level developing in the shorter cycles is in good agreement with the results of TAKÁCS and MARÓTI (1983), and NAYLOR and GILES (1982), who found fewer amount of starch granules in chloroplasts in the case of cycle lengths between 30 and 5 min., than in chloroplasts of plants grown under continuous light.

From the new LDP-s to which the plants were transfered at the age of 22 days, the shorter decreased, and the longer increased the carbohydrate level; both in the case of starch and soluble sugars. That is, the second LDP (from the age of 22 to 37 days) had determinative effect on the carbohydrate level measured at the age of 37 days. Thus, for example, the carbohydrate level of the leaves placed from the 16–8 hours LDP to 15–7.5 min. LDP was found to be close to equal to the leaves receiving 15–7.5 min. LDP treatment throughout the experiment, and the carbohydrate level of the 4th leaf of the plants transfered from 15–7.5 min. LDP to 16–8 hours LDP also came close — although not reaching — that of the equivalent leaves exposed throughout to 16–8 hours LDP-s. It seems that the short periodical illumination caused a disturbance in the carbohydrate metabolism and even the plants placed to longer LDP at 22 days of age were incapable of complete "regeneration".

The developing leaves were also characterized by the above mentioned alterations, only in a slighter degree.

The determination in regard to the evaluation of the dryweight is also valid for the carbohydrate level, according to which the observed alterations were the most striking in the case of the combination of the 16–8 hours and 15–7.5 min. LDP-s, being similar but of decreased degree in the case of the other two combinations.

Discussion

The cellular biochemical and fine structural interpretation of the differing effects of alternating LDP-s is still scant (NAYLOR and GILES, 1982). Several theories are known: "photosynthetic saturation" (EMERSON and ARNOLDS, 1932), "endogeneous daily rhythm" (BÜNNING, 1935, 1950), "phytochrome control" (KIGEL and KOLLER, 1970; SCHOPFER, 1972; HURD, 1973), but in our opinion the more profound cause is unknown as yet regarding the effect of alternating LD periods.

It has been demonstrated (MARÓTI and PATAKY, 1982; MARÓTI and MIHALIK, 1982) that the dry matter-content increasing and decreasing effect, resp. of alternating LD periods (besides constant light intensity and identical daily amount of illumination) is determined by the length of the light, dark periods and by the genotype.

According to SAGER and GIGER (1980) the photosynthetic efficiency of the alternating short light-dark periods only rarely surpasses the value measured in continuous light. The rarity is disputable since several experiments are known in which the plants produce more dry-matter in the shorter LDP-s than in continuous light (GARNER and ALLARD, 1931; EMERSON and ARNOLD, 1932; BONDE, 1955; POLLARD, 1970; HORVÁTH and MIHALIK, 1977; MARÓTI and PATAKY, 1982, and others).

It is very likely that under cell level the alternating LDP-s firstly have effect in the chloroplast. In the short LDP-s the chloroplasts either swell or shrink, depending on the "genotype" and the length of the periods. The change in the volume of maize chloroplasts is in negative correlation with the dry-matter production in mesophyll cells, and in positive correlation in bundle sheath cells (MARÓTI and PATAKY, 1982). Concerning the structure of the chloroplasts, in the case of beans the characteristic effect of the 30–15, and 15–7.5 min. LDP-s is expressed by the almost complete lack of starch from the chloroplasts, the decrease in the thylakoid number per grana, the significant increase per unit surface of the stroma lamellae and end membrane length (TAKÁCS and MARÓTI, 1983).

The currently performed electronmicroscopic studies on the P 3839 maize (PATAKY, personal communication) also show that the organization of the inner membrane of chloroplasts is responsible for the fact that the maize grown earlier in short (30–15, and 15–7.5 min.) LDP make better use of the light in 16–8 hours LDP than the one, kept throughout in short or longer LDP-s.

According to our assumptions the varying effect of the short LDP-s at such a weak light intensity (32 W/m^2) is not determined by the dark, but firstly by the light reactions, and is in direct relationship with the speed of the proton-accumulation in the loculus of the thylakoid, as well as with the development in time of the electron stream of inductive phase (MARÓTI, 1982).

The effect of the alternating LDP-s above cell level may firstly be in relationship with the structure, size, carbohydrate content of the leaves and the trend and degree of the translocation. The size of the leaf area is in tight correlation with the carbohydrate content of the leaves (BOOYSEN and NELSON, 1975).

In our experiments, too the prominent dry-matter amount was accompanied by high soluble sugar and starch content in the LDP-s having favourable effect on growth.

According to the studies of NAYLOR and GILES (1982) carried out on beans the decrease in dry-weight is joined by the increase in the quota of roots in the case of

short LDP-s. This was not experienced by us in the 15–7.5 min. LDP, at the same time the placing of the plants from short LDP to longer LDP presented such an increase in the dry-matter quota of the roots which had only slight decreasing effect on that of the leaves.

References

BONDE, E. K. (1955): The effect of various cycles of light and darkness on the growth of tomato and cocklebur plants. — Physiol. Plant. *8*, 913–923.

BOOYSEN, P. and NELSON, C. J. (1975): Leaf area and carbohydrate reserve in regrowth of tall fesuce. — Crop. Sci. *15*, 262–266.

BÜNNING, E. (1935): Zur Kenntnis der Endogenen Tagesrhythmik bei insekten und bei Pflanzen. — Ber. D. Bot. Ges. *53*, 594–623.

BÜNNING, E. (1950): Über die photophile und scotophile phase der endogenen Tages-rhythmik. — Planta *38*, 521–540.

DUBOIS, M., GILLES, K. A., HAMILTON, J. K., ROBERTS, P. A. and SMITH, E. (1956): Colorimetric method for determination of sugar and related substances. — Anal. Chem. *28*, 350–356.

EMERSON, R. and ARNOLD, W. (1932): A separation of the reactions in the photosynthesis by means of intermittent light. — J. Gen. Physiol. *15*, 391–420.

GARNER, W. W. and ALLARD, H. A. (1931): Effect of abnormally long and short alternations of light and darkness on growth and development of plant. — J. Agric. Res. *42*, 629–53.

HORVÁTH, I. (1972(: Phytotron in the Botanical Garden of the József Attila University, Szeged. — Acta Biol. Szeged. *18*, 15–19.

HORVÁTH, I. and MIHALIK, E. (1977): The effect of the rhythm of illumination on the accumulation of dry-matter. — Bot. Közlem. *64*, 121–124.

HURD, R. G. (1973): Long day effect on growth and flower initiation of tomato plants in low light. — Ann. appl. Biol. *73*, 221–228.

KAISER, W. M. and BASSHAM, J. A. (1979): Light-dark regulation of starch metabolism in chloroplasts. — Plant Physiol. *63*, 109–113, and 105–108.

KIGEL, K. and KOLLER, D. (1970): Analysis of the control of development in *Orysopsis miliacea* by the light enviroment. — J. Exp. Bot. *21*, 1003–1016.

MARÓTI, I. (1982): Effect of light-dark cycles on the chlorophyll and carotenoid content of maize and tomatoes, — Acta Biol. Szeged. *28*, 86–94.

MARÓTI, I. and MIHALIK, E. (1982): The effect of short periodical illumination on the dry-weight, soluble sugar, strach and cellulose contents of maize- — Bot. Közlem. *69*, 259.

MARÓTI, I. and TAKÁCS, E. (1983): The effect of the short rhythm illumination on the lamellar system of the *Zea mays* mesophyll schloroplasts. — Acta Biol. Szeged. *29*, MARÓTI, I. and PATAKY, SZ. (1982): Effect of alternating light-dark cycles on the size of maize chloroplasts and on the accumulation of dry matter. — Acta Biol. Szeged. *2* , 95–109.

NAYLOR, W. A. and GILES, L. J. (1982): Growth, pigment synthesis and ultrastructural responses of *Phaseolus vulgaris* L. cv Blue Lake to intermittent and flashing light. — Plant Physiol. *70*, 257–263.

POLLARD, D. F. W. (1970): The effect of rapidly changing light on rate of photosynthesis in large-tooth aspen *(Populus grandidentata)*. — Canad. J. Bot. *48*, 823–828.

PREISS, J. (1982): Regulation of the biosynthesis and degradation of starch. — Ann. Rev. Plant Physiol. *33*, 431–454.

SAGER, J. C. and GIGER, W. J. R. (1980): Re-evaluation of published data on the relative photosynthetic efficiency of intermittent and continuous light. — Agric. Meteorol. *22*, 289–302.

SCHOPFER, P. (1972): Phytochrome and the control of enzyme activiti. In MITRAKAS, K. and SCHROPSHIRE, W. ed.: Phytochrome pp. 483–514. Acad. Press London.

TAKÁCS, E. and MARÓTI, I. (1983): The effect of short light-dark cycles on the membrane sytem of bean chloroplasts. — ○ ○ ○

Address of the authors
DR. I. MARÓTI
DR. KATALIN MARGÓCZI
Department of Botany, A. J. University,
H-6701 Szeged, P.O. Box 428.
Hungary

Acta Biol. Szeged. 30, pp. 61–73 (1984)

THE EFFECT OF SHORT LIGHT-DARK CYCLES ON THE MEMBRANE SYSTEM OF BEAN CHLOROPLASTS

EDIT TAKÁCS and I. MARÓTI

Attila József University, Department of Botany, Szeged

(Received June 30, 1983)

Abstract

The effect of alternating 30–15, and 15–7.5 min. light-dark cycles (LDC) as well as of 16 hours long-day illumination and 8 hours dark were studied on 4 week old Valja bean grown in phytotron. In every case the daily period of illumination was 16 hours, the light flux density was 32 Wm^{-2}, the temperature was 21 ± 1 °C.

The short rhythms were unfavourable for the development of beans. Compared to the 16–8 hours LD control the accumulation of dry-matter decreased, necrotic patches appeared on the yellowishgreen leaves. The characteristic effect of the short LDC-s on the structure of the chloroplasts was that the starch was almost completely missing from the plasts, the thylakoid number per grana decreased, the stroma lamellae and end granal membrane lengths per unit surface considerably increased. The ratio of the grana composed of 2–5 thylakoids manifested four-five times increase in the 30–15 and 15–7.5 min. LDC-s compared to the 16–8 hours control. The granal intrathylakoidal spaces expanded. In one part of the plasts the membranes of the columnar grana became swollen and disorganized.

Key-words: light-dark cycles, chloroplast, grana, stacked membranes, unstacked membranes, dry matter, bean.

Introduction

The length and proportion of light-dark periods (LDP) significantly change the pigment-content, membrane structure of chloroplasts, the pH relations of the intrathylakoidal space and stroma plasma (MARÓTI, 1982; MARÓTI and PATAKY, 1982). The 30–15, 15–7.5 min. alternating short LDP-s imply strong "light-dark stress" on the development of several plants. They become yellowish-green, the chlorophylls and carotenoids show significant and specific disorganization, necrotic patches appear on the leaves.

Depending on genotype, the dry-matter production either increases or decreases.

The length of the dark period and the ratio of light-dark, resp., have great effect on the membrane organization of chloroplasts (ARMOND et al., 1976; DAVIS et al., 1976; AKOYUNOGLOU and ARGYROUDI—AKOYUNOGLOU, 1978).

In greening experiments where the light-dark ratio varies between 1/10–1/90 — ie. the rather short (0.01 sec. — 2 min.) and short (2–15 min.), resp. light periods are followed by long dark periods — characteristic changes are observable: no normal grana are formed, only so-called double primary thylakoids. The ratio of chlorophyll a/b is high. The oxygen evolution is hindered (STRASSER and BUTLER, 1976). The cation regulation of the chlorolpasts is decreased. Less chlorophyll-protein complexes

are formed than in continuous illumination (ARGYROUDI—AKOYUNOGLOV et al., 1971; SÁRVÁRI and GEIGLER, 1982).

The effects of the short (30–15 and 15–7.5 min.) 2/1 proportioned light-dark periods applied by us essentially differ from the above; normal grana develop, the ratio of chlorophyll a/b is only slightly higher than in the 16–8 hours control LDP, the plants flower and produce. The changes in the membrane system of the chloroplasts are generally not so drastic as in the case of short light-long dark treatments. Therefore, our electronmicroscopic studies serve with data to the relationship between the membrane structure and utilization of light.

The present paper reports on the changes of chloroplasts of the 3rd leaf of 4 week old beans grown in 30–15 and 15–7.5 min. LDP-s, compared to the 16–8 hours control. Attempt was made firstly to correlate the degree of thylakoid aggregation, the amount and ratio of stacked and unstacked membranes with dry-matter production.

Material and Methods

The studies were performed on *Phaseolus vulgaris* L. c. v. Valja beans. The plants were grown in phytotron (HORVÁTH, 1972), in the mixture of sand-perlite 1:1, with nutrient of HOAGLAND (REYSS and BOURDU, 1970). The moisture content of the soil was 70% of the water capacity. The nutrient suplly was ensured by 20 ml nutrient solution twice a week, the original humidity of the soil was maintained by daily watering with distilled water. The daily amount of light was the same. The control plants were grown in 16 hours continuous illumination and 8 hours dark. In the short-rhythm experiments alternating periods of 30 min. light, 15 min. dark, and 15 min. light, 7.5 min dark were applied. The light flux density was 32 Wm^{-2} in the climate chambers, using F_{29} light tubes. Table 1 comprises the spectral energy dispersion of the light tubes. The plants were grown at constant temperature of 20 ± 1 °C.

For electronmicroscopic studies the samples were taken from the central part of the lateral leaflet of the 1. tripartite leaf at the age of 4 weeks. The leaf pieces with diameters of 0.5–1 mm were fixed in 3% glutaraldehyde, contrasted with 2% $KMnO_4$ solution, dehydrated in ethylalcohol series, and embedded into Durcupan ACM resin. The sections prepared with Reichert ultramicrotome were stained with Pb citrate.

The pictures of the chloroplasts of the mesophyll palisade parenchyma were prepared by Tesla BS 212 E and Tesla BS 500 electronmicroscope. The planar size of the plasts, the number and size of the grana, the grana/thylakoid number as well as the length and ratio of stacked and unstacked membranes were measured on pictures of known magnification. 30–40 plasts were evaluated per treatment.

Table 1. Energy dispersion of F29 light tubes in the % of energy falling to the 400-700 nm wave domain.

Violet 400–436 nm	Blue 436–495 nm	Green 495–560 nm	Yellow 560–589 nm	Orange 589–627 nm	Red 627–700 nm
2.25	7.38	18.06	22.87	29.21	20.23

Results

In the experiments both the 30–15 min. and 15–7.5 min. light-dark periods decreased the production of the plants. As the results of the short periodical illumination the total dry-matter production decreased to about the half. In tendency the organic dry-weight followed the trend of the total weight.

The dry-matter accumulation was the lowest in the 30–15 min. LDP, being only 40% of that of the control plants. This is firstly the consequence of the more considerable reduction in stem and leaf weights (Table 2).

Table 2. Dry-matter production (related to 1 plant). The data are the average values of 25–25 plants per treatment. Processing was accomplished at four weeks of age.

Light treatment	Dry weight mg/plant						
	root	stem	primordial leaves	1. tripartite leaves	2. tripartite leaves	3. tripartite leaves	total
16–8 hours LDP	332	273	63	129	116	98	1012
30–15 min. LDC	133	141	39	48	49	56	466
15–7.5 min. LDC	137	179	33	62	55	74	540

The size of the chloroplasts of the palisade parenchyma was enhanced by the short light-dark cycles (Table 3). The shape of the chloroplasts changed; they became elongated and flattened.

A large amount of starch was found in the chloroplast of plants exposed to long-day (16–8 hours) LDP. 35% of the section-surface of the chloroplasts contained starch, the membrane system was found to aggregate at the peripheral areas (Plate I, Table 1 and 3).

Table 3. The effect of short periodical treatment on the structure of chloroplasts. The plasts of the palisade parenchyma of the 1. tripartite leaves were evaluated. The data are the average values of 30–40 chloroplasts.

Light treatment	Chloroplast section area (μ^2)	Proportion of area %		Number of grana (10 μ^2)	Stroma lamellae (μ/μ^2)	Partition leghth (μ/μ^2)	End granal membrane (μ/μ^2)	Proportionof stroma lamellae to total lamellar material (%)	Proportion of partition to total lamellar material (%)	Proportion of end granal membrane to total lamellar material %
		starch	grana							
16–8 hrs LDP	11	30	22	25	1.86	3.19	1.16	30	52	18
30–15 min. LDC	19	2	13	11	3.41	3.32	1.78	40	39	21
15–7.5 min. LDC	18	2	13	21	2.78	4.09	2.64	30	43	27

The assimilatory starch did not accumulate in the chloroplasts of bean leaves grown in short periodical illumination. The amount and surface ratio of the grana (Table 3).

The number of grana per section-surface increased on the effect of 15–7.5 min. LDP-s and decreased on that of 30–15 min. periodical illumination. Converted into unit surface the degree of decrease in the amount of grana (more than 50%) was rather significant in the 30–15 min. light-dark periods.

In the leaves grown under continuous 16 hours illumination the surface ratio of the grana amounted to about 22% of the cut-surface of chloroplasts. The area of grana was reduced to nearly the half by the short cycles, from the whole sectionsurface the quota of grana was 13% in the case of both LDC-s. The effect of short periodical illumination on the shape of grana and the amount of grana/thylakoid (Fig. 1).

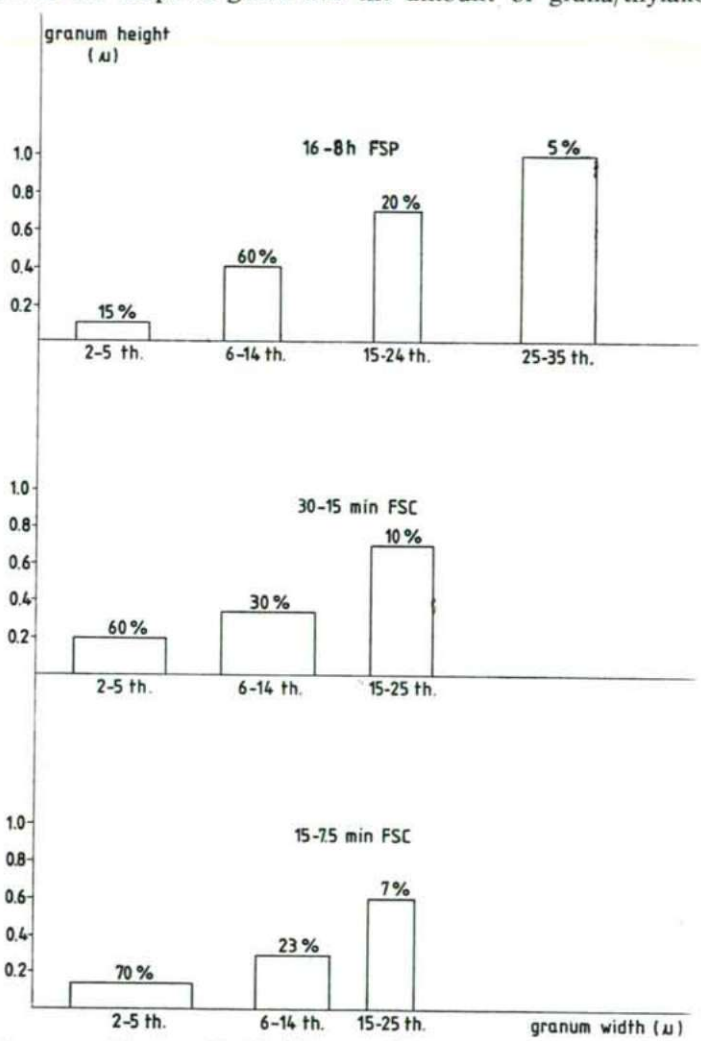


Fig. 1. Size of grana and degree of thylakoid aggregation.

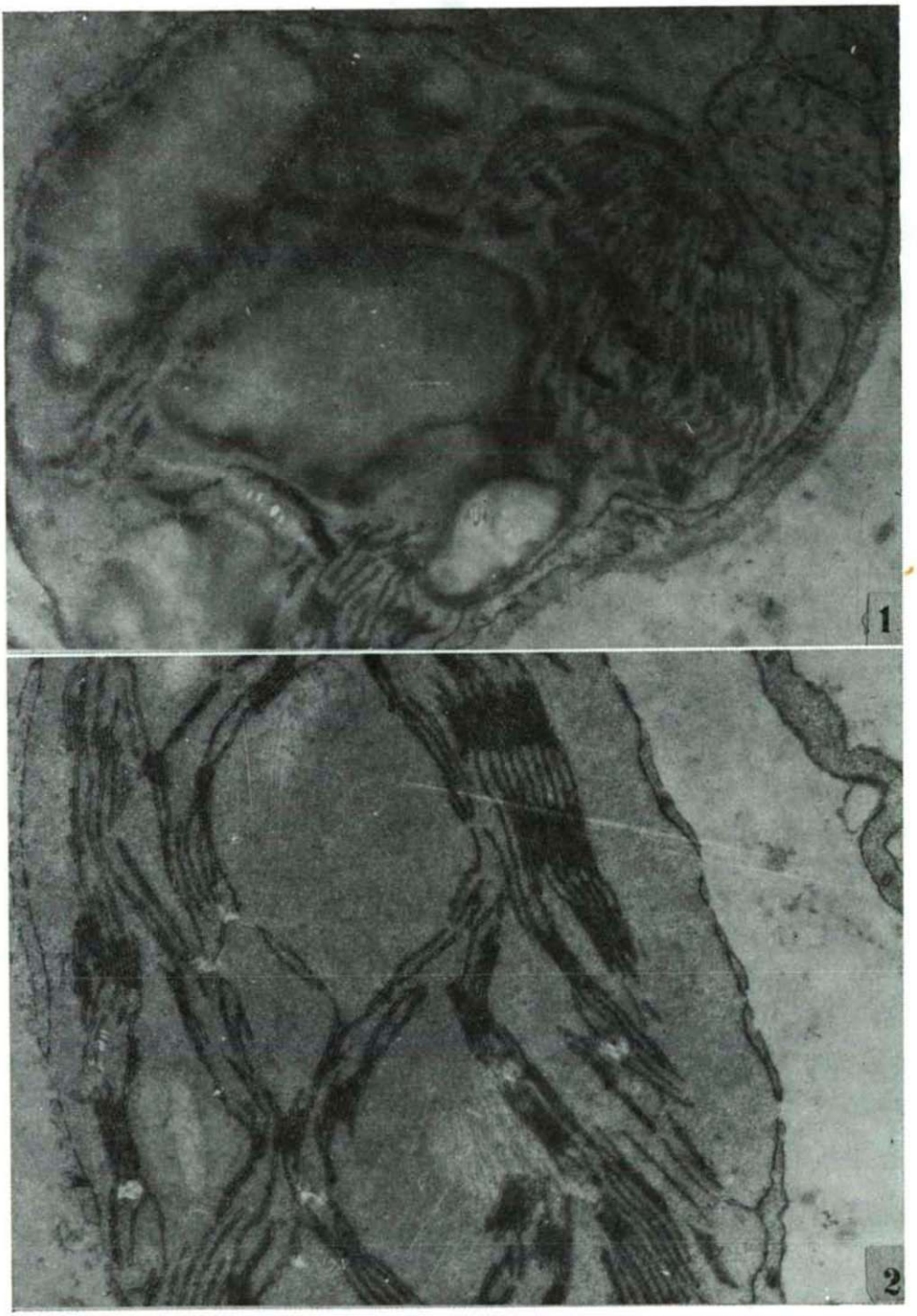


Plate I. 1. light-dark cycle 16–8 hrs (25 000 X), 2. light-dark cycle 30–15 min (25 000 X).

a. The size, height and width of the grana

On the basis of the thylakoid aggregation the grana were separated into 4 groups: standing of 2–5; 6–14; 15–24, and 25–35 thylakoids.

The percental distribution of these four groups was also evaluated.

In the chloroplasts of leaves growing in continuous light the grana made up of 6–14 thylakoids were dominating, giving 60% of the total amount of grana.

Due to the multiple adherence of the thylakoids the frequency of the grana composed of 15–35 compartments was 25% in the control chloroplasts. The grana having 2–5 thylakoids occurred in lowest number, their shape differed from the rest, the diameter of their width surpassed their height measures.

Regularity, tight correlation was observed between the degree of thylakoid aggregation and the grana shape. The partition of the multithylakoidal grana was short, the height/width ratio was high. With the decrease of thylakoid aggregation the adhering surface of certain grana compartments became larger.

On the effect of the periodical treatment, there was a change in the membrane organization of the chloroplasts.

The uniform effect of the short rhythms was that the 25–35 thylakoidal, columnar macrograna did not develop and the ones standing of 2–5 thylakoids occurred in 50–70%.

Chloroplasts of varying membrane structure developed on the effect of 30–15 min. light-dark cycles. The heterogeneous plasts could be divided into three groups. Only one kind of chloroplast could be found within each palisade cell.

1. The chloroplasts having granum structure, similar to those of the long-day illumination occurred in the highest amount, forming 55–60% of the studied plasts (Plate I/2). Less high and wider grana developed in these chloroplasts. The 2–5 thylakoidal grana were the most frequent; 60% of the total amount of grana.

The grana joined by 5–14 compartments were also frequent (30%), and the ones containing 15–25 thylakoids occurred in about 10%.

The average thylakoid number of the grana decreased to almost the half compared to the control. The smaller degree of aggregation was compensated by higher partition. The shape of the grana made up of similar amount of thylakoids changed in the 30–15 min. LDC, the partitions became longer (Fig. 1).

2. In one part of the chloroplasts (25%) the grana intrathylakoidal spaces widened. The loculi found at the end granal membranes dilated into ducts having wide cavities and at many places into wide sacks. The cavity of the thylakoid loculi also varied within the grana. Many vesicules could be found at the end of the stroma lamellae, furthermore the interlamellar spaces also strongly widened (Plate II/1). The degree of aggregation of the thylakoids and the value of the grana surface ratio were similar to that of the previous group.

Thus, destructive processes appeared in the chloroplasts on the effect of the 30–15 min. LDC-s, becoming the general characteristics of the chloroplasts. On the effect of treatment the terminal vesicules of the grana, being contiguous with the stroma became swollen, the detached wall of the vesicules showed undulatory contour.

The swelling not only appeared in the case of the vesicules having terminal location, but also in the stroma thylakoids, the diameter of the isolated, long lamellae was also dilated (Plate II/3). These processes of swelling were not characteristic to every chloroplast membrane, however, these manifestations commenced in 25%

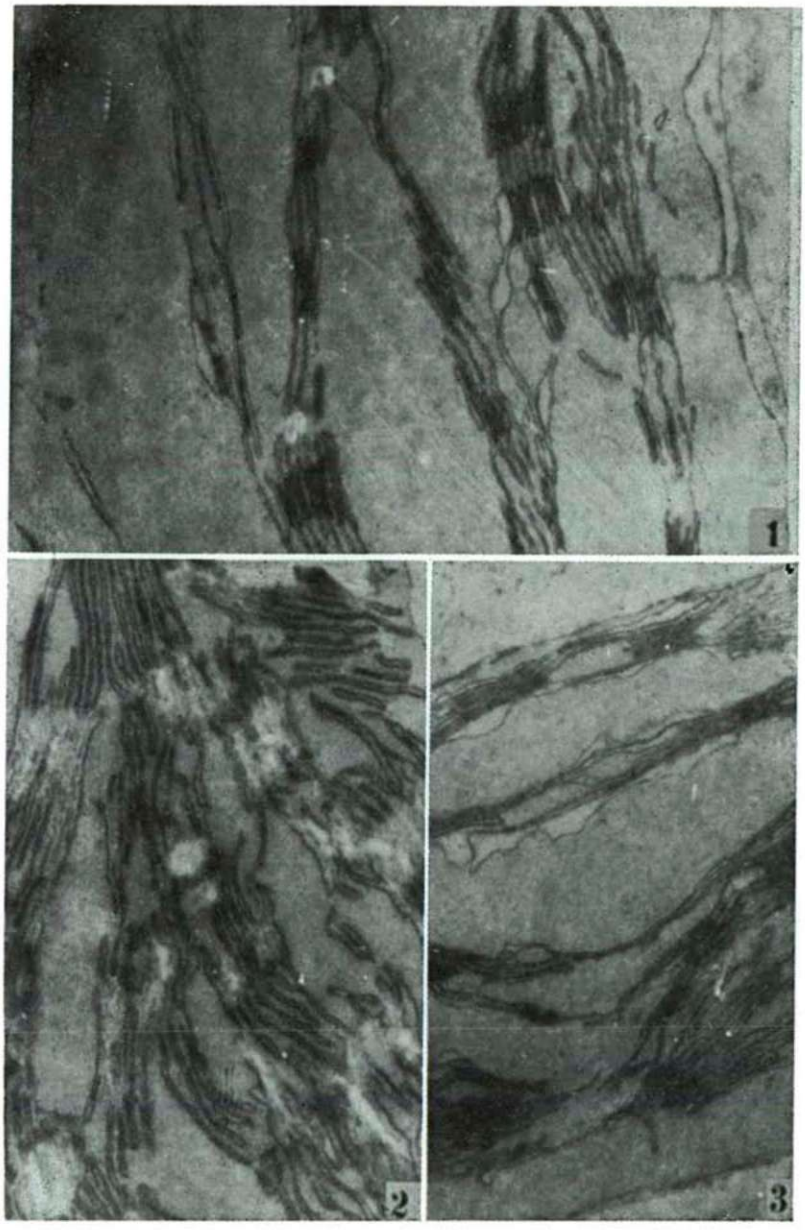


Plate II. 1. light-dark cycle 30–15 min (25 000 X), 2. light-dark cycle 30–15 min (25 000 X), 3. light-dark cycle 30–15 min (25 000 X).

of the studied chloroplasts, to a lesser or higher degree. The sporadical, short stroma lamellae took up undefined shape and undulatory contour.

3. In close to 20% of the studied chloroplasts the columnar grana became completely swollen, the membranes became resolved. In the chloroplasts contrasted with $KMnO_4$ the spaces of the grana appeared as white patches, the chloroplasts showed mosaic appearance. In these chloroplasts the stroma lamellae dominated. It is presumably the destructive effect of the dark periods of the short cycles which manifested, even affecting destructively the intact, developed chloroplasts (Plate II/2).

The 15–7.5 min. LDC-s considerably decreased the average thylakoid number of the grana compared to the 16–8 hours long-day illumination. The significant reduction in thylakoid aggregation was shown by the 70% occurrence of the 2–5 thylakoidal grana. Compared with the control the grana were wider, the adhering surface of each grana disc became larger. The grana made up of 15–22 thylakoids occurred in 7% (Plate III/1).

The granal intrathylakoidal spaces became dilated, the expansion of the loculi was firstly detectable in the case of the therminal grana thylakoids. Decomposition could not be observed in the membranes of the grana (Plate III/2).

b. Ratio of Stacked and unstacked membranes

The light-dark cycles substantially changed the membrane organization of the chloroplasts.

The stacked membranes (partition) and the unstacked membranes (stroma lamellae+end membranes) were evaluated in respect to their lengths falling to unit surface and their proportion regarding the total membrane lengths.

In the 16–8 hours treatment the ratio of stacked. membranes was strikingly high, the thylakoid membranes were made up of partitions in more than 50%. The proportion of the end granal membranes was 18% to the total membrane lengths, and that of the stroma lamellae was 30%. Even concerning unit area the length of the partition membranes was the greatest.

In 30–15 min. light-dark periods the ratio of the stacked and stroma lamellae was identical (40%). The distribution of the grana standing of few (2–5) and many (7–22) thylakoids was found to be close to similar.

The ratio of the unstacked membranes (stroma+ end membrane) was 60%, being higher than that of the partitions (40%). Compared to the control a significant increase was experienced in regard to the ratio of the stroma lamellae, this being of slighter degree in the case of the end granal membranes.

The end granal membrane lengths regarding surface unit increased from 1,1 μ to 1,8 μ, since the grana became wider with the lower degree of aggregation. At the same time the percental quota of the stacked membranes decreased from the total membrane length.

The value of the total membrane length falling to 1 μ^2 was higher in the case of the 30–15 min. LDC than in the control plasts; in the latter the value of lamellation per unit area was 6 μ, which increased to 8,5 μ in the 30–15 min. LDC.

Compared to the 16–8 hours long-day illumination the 15–7.5 min. light-dark cycle significantly increased the ratio of end granal membranes on the one part (by 10%), on the other part it decreased the percental quota of the partition — also by 10%.

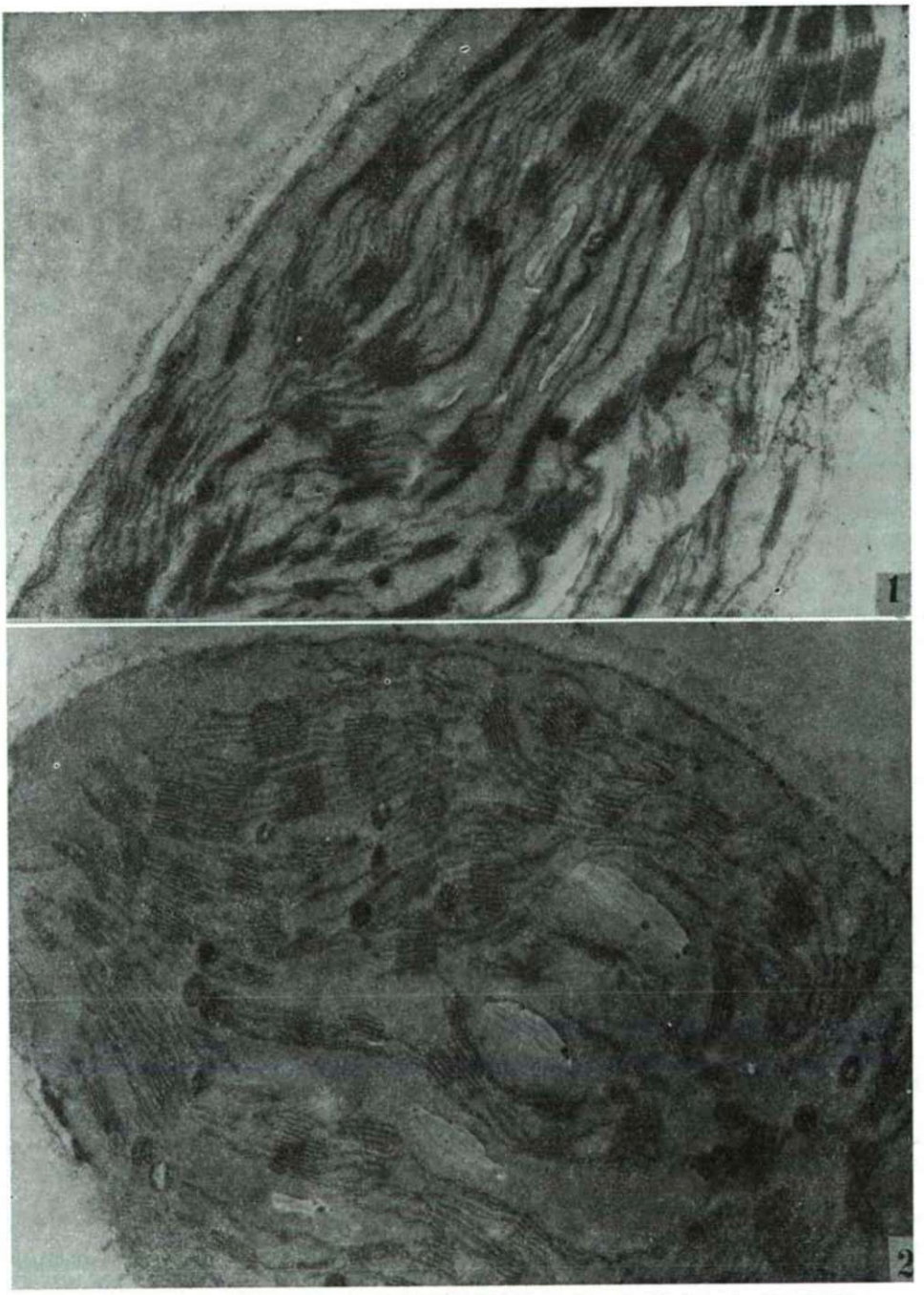


Plate III. 1. light-dark cycle 15–7.5 min (25 000 X) 2. light-dark cycle 15–7.5 min (25 000 X).

The partition length regarding unit area of the plasts surpassed the value of the other two light-treatments. The length of the total membrane system observed at an area of 1 μ^2 showed maximal values in the case of the 15–7.5 min. LDC. It was the surface of the end granal membranes which increased in the highest degree, surpassing twice the values of the control plants. This alteration could be explained by the increase in the amount of grana.

Discussion

The effect of light-treatments different from the natural rhythm — in the case of identical light intensity and illumination periods — is determined by the length of the light-dark periods.

The control plants as well as those treated with light-dark periods received light of the same intensity. The daily total energy amount was also identical. Despite this, the alternating light-dark cycles resulted changes differing from the control. The rhytmic illumination caused a change in the photosynthetic structure.

The utilization of light depends fundamentally on the organization of the chloroplast's membrane system, the size of the membranes, the degree of thylakoid aggregation, and the ratio of unstacked and stacked membranes.

The light-dark cycles varying between 1 to 30 munites have in general unfavourable effect on the development of the plants. In these cycles the leaves become yellowish-green, and the appearance of necrotic patches can be detected. There is a decrease in the accumulation of dry-matter, the chlorophylls and carotenoids become significantly decomposed, and in the latter stage of ontogenetic development the leaves become white as the consequence of destruction regarding the photosynthetic apparatus.

The 30–15 min. LDC-s have decreasing or increasing effect on the dry-weight of the plants — depending on the type. The fact can be evidenced best in the case of this cycle that in the light-dark periods of lengths from 5 min. to 6 hours the photosynthetic utilization of light varies according to types (FÜLÖP, 1982; MARÓTI and MIHALIK, 1982; MARÓTI and PATAKY, 1982).

In the case of the Valja bean both short cycles decrease the total production of the plants. The leaf production decreases to smaller extent in the 15–7.5 min. LDC; the dry-weight of the leaves is higher in this cycle than in the 30–15 min. LDC.

It is our assumption that the degradation of the dry-matter is not caused by the accumulation in primary products, but by the destruction of the chloroplasts.

The effect of the light-treatments on the structural state of the plasts is also well shown by the main product of the chloroplasts, the starch. The starch found in high amount and huge proportions in the plasts of the control leaves refers to the intensive course of photosynthesis. Active starch synthesis can be manifested, the formed enormous starch grains fill out 30% of the chloroplast volume. In the leaves grown under short cycles only low amount of starch occurs in the chloroplasts (1–2%).

The question arises, what relationship exists between the changes in the shape, amount and area ratio of the grana and the distribution of the adhered and single membranes?

The number of grana was altered differently by the two kinds of short cycles. A decrease was observable in the 30–15 min. LDC, while in the 15–7.5 LDC the

granum number per cut surface was 30% more than in continuous light, and the area ratio decreased to almost the half. The area ratio of the grana depends on the number of grana and grana vesicules. In the 30–15 min. light-dark cycle the area ratio of the grana decreased proportionally with their amount.

In one part of the chloroplasts of the plants grown under this cycle the structural destruction was irreversible, the grana thylakoids became completely decompodes, whole grana "disappeared"; here the depression of the photosynthetic CO_2 assimilation is presumably of higher degree which could also be evidence in the smallest leaf product.

The highest amount of grana developed in the 15–7.5 min. LDC, the large grana became disjointed, the quota of the smaller grana increased. The fact that the number of grana increased by 30% compered to the control, and their quota from the cut surface decreased almost to the half is partly the result of the change also in the size and shape of the chloroplasts. The length per width ratio of the chloroplasts increased since they became elongated and flattened. The smaller part of the larger plasts was occupied by the grana. On the other hand, there was also a decrease in the size of the grana. Firstly the increase of the stroma plasma was observable. The degree of chloroplast-organization was followed with attention by the values and distribution of the grana and stroma lamellae, as well as the stacked and unstacked membranes, resp.

The flattening of the chloroplasts is also in connection with the thylakoid number per granum. The ratio of stacked and unstacked membranes shifted to the benefit of the unstacked membranes (stroma + end granal membrane) in the case of the short cycle plasts. This is significant in the 15–7.5 min. LDC, where there are more grana and the end granal membranes are longer.

The end granal membrane length falling to 1 μ^2 and the quota of the end membranes from the complete membrane system are the greatest; as well as the length of partition per unit area.

The lamellar system is the most developed in the 15–7.5 min. LDC from the three light-treatments, and this is where the total thylakoid length per 1 μ^2 is the largest.

Studying the relationship between the light utilization and the membrane system of the chloroplasts (BJÖRKMAN, 1975; BOARDMAN et al., 1974; LICHTENTHALER, 1981; PRENZEL et al., 1980) it becomes evident from the works of the listed authors that the formation of grana is greatly influenced by the intensity of illumination. Low light intensity or reduced radiance is favourable for the development of grana, and the surface of membranes per unit area is rather large.

The structure of the chloroplasts developing on the effect of the short cycles applied in our experiments is similar to the shade type chloroplasts, in so far as no starch is accumulated, the grana are wider, less grana can be found per unit surface, and the total thylakoid length per unit area is rather high.

There are sifferences, however, in regard to the degree of thylakoid aggreation, as the adnating percentage of the thylakoids is lower.

In the rhytmic chloroplasts the ratio of stroma lamellae and end granal membranes increased from the whole membrane system, the efficiency of photosynthetic quantum-transformation decreased. In the 30–15 min and 15–7.5 min. LDC-s the alternation of the light-dark periods inhibits photosynthetic electron stream and weakens CO_2 fixation.

Correlation was sought between the abnormal chlorophyll a/b ratio and the structural anomalies; that is, to what extent does the utilization of light depend on the development of the chloroplast membranes?

One of the characteristic effects of the short periodical illumination is that there is a decrease in the area ratio of the grana.

It is known that there is tight correlation between the production and granal thylakoid number (HORVÁTH and MIHALIK) 1978,

Analysing the results, it could be determined that the damaging effect of the short light-dark periods is firstly manifested in the development of grana having fewer amount of thylakoids, the partition of which is higher.

Destruction of larger degree leads to structural changes in the grana vesicules. This was experienced in the 30–15 min. LDC where the grana vesicules became swollen, then the thylakoids became decomposed.

These morphological alterations also cause considerable decrease in the amount of chlorophylls and carotenoids. The chlorophyll-content shows positive correlation with the number of grana vesicules found in the chloroplasts.

The change in the amount of chlorophyll-b evidences the effect of the treatments the best. Certain plants react in various degrees; in bean and mustard leaves the amount of chlorophyll-b decreases to a large extent, therefore there is an increase in chlorophyll a/b. Outstanding decrease is observable in the amount of violaxanthine, too (AL-SUBAI, 1980).

The considerable decrease in xanthophylls and chlorophyll-b may also be the cause of the relative instability of the LHCP.

The degradation of the chlorophyll-proteins is in connection with the decomposition of the thylakoids (LICHTENTHALER et al., 1981). The chlorophyll-protein complex becomes defected in the fiest place, which is in connection with the PSII. The significant accumulation of the 2–6 thylakoidal grana could be explained by the fact that due to the short and frequent light-dark periods the cation/proton ratio on the stroma plasma and intrathylakoidal space often changes, and differs from the continuous light, resp., which alteration may also affect the chlorophyll-protein complexes participating in the adherence.

The general effect of the short cycles studied by us is that they decrease the amount of starch and are also unfavourable to the accumulation of the total carbonhydrate and dry-matter (MARÓTI and MIHALIK, 1982).

References

AKOYUNOGLOU, G. and ARGYROUDI-AKOYUNOGLOU, J. H. (1978): Control of thylakoid growth in Phaseolus vulgaris. — Plant Physiol. *61*, 834–837.

AL-SUBAI, M. Y. (1980): The effect of rhythmic illumination on the organic matter production and tissue structure of plants. Dissertation for Candidate Degree, Theses.

ARGYROUDI-AKOYUNOGLOU, J. H., FELEKI, Z. and AKOYUNOGLOU, G. (1971): Formation of two chlorophyll-protein complexes during greening of etiolated bean leaves. — Biochem. Biophys. Res. Commun. *45*, 606–614.

ARMOND, P. A., ARTZEN, C. J., BRIANTAIS, J. M. and VERNOTTE, C. (1976): Differentiation of chloroplast lamellae. Lightharvesting efficiency and grana development. — Arch. Biochem. Biochys. *175*, 54–63.

BJÖRKMAN, O. (1975): In environmental and biological control of photosynthesis (Ed.: MARCELLE, R.) 1–16. W. Junk, The Hague.

BOARDMAN, N. K., BJÖRKMAN, O., ANDERSON, J. M., GOODCHILD, D. J. and THORNE, S. W. (1974): Photosynthetic adaptation of higher plants to light intensity: relationship between chloroplast structure, composition of the photosystems and photosynthetic rates. Proceedings of 3rd Int. Cong. on Photosynthesis (Ed.: AVRON, M.) Amsterdam: Elsevier, 1809–1829.

DAVIS, D. J., ARMOND, P. A., GROSS, E. L. and ARNTZEN, C. J. (1976): Differentiation of chloroplast lamellae onset of cation regulation of excitation energy distribution. — Arch. Biochem. Biophys. *175*, 64–70.

FÜLÖP, E. (1982): The effect of short light-dark cycles on the morphology of maize and tomatoes, and the tissue structure of the leaves. Doctoral Dissertation.

HORVÁTH, I. (1972): Phytotron in the Botanical Gardens of the József Attila University, Szeged. — Acta Biol. Szeged. *18*, 1–4.

HORVÁTH, I. and MIHALIK, E. (1978): The effect of illumination of changing rhythm upon the organic-matter production and energy utilization of mustard. — Bot. Közl. *65*, 181–186.

LICHTENTHALER, H. K. (1981): Adaptation of leaves and chloroplasts to high quanta fluence rates. Photosynthesis VI. Photosynthesis and productivity, Photosynthesis and Environment. 273–287.

LICHTENTHALER, H. K., BURKARD, G., KUHN, G., and PRENZEL, U. (1981): Light- induced accumulation and stability of chlorophylls and chlorophyllproteins during chloroplast development in radish seedlings. — Z. Naturforsch. *36*, 421–430.

MARÓTI, I. (1982): Effect of short light-dark cycles on the chlorophyll and carotenoid content of maize and tomatoes. — Acta Biol. Szeged. *2* , 85–94.

MARÓTI, I. and MIHALIK, E. (1982): The effect of short periodical illumination on the dry-weight, soluble sugar, starch and cellulose contents of maize. First Congress on Plant Physiology, Szeged.

MARÓTI, I. and PATAKY, Sz. (1982): Effect of alternating lightdark cycles on the size of maize chloroplasts and on the accumulation of dry matter. — Acta Biol. Szeged. *28*, 95–110.

PRENZEL, U., LICHTENTHALER, H. K. and MEIER, D. (1980): In advances in the biogenesis and function of plant lipids. Amsterdam.

REYSS, A. and BOURDU, R. (1971): Influence des héméroperiodes trés courtes sur la croissance de Lolium multiflorum, sa composition pigmentaire et ultrastructure chloroplastique. Planta (Berlin) *97*, 230–244.

SÁRVÁRI, É. and GEIGLER, G. (1982): Chlorophyll-protein complexes in seedlings treated with lincomycin. First Congress on Plant Physiology. Szeged.

STRASSER, R. J. and BUTLER, W. L. (1976): Correlation of absorbance changes and thylakoid fusion with the induction of oxygen evolution in bean leaves greened by brief flashes. Plant Physiol. *58*, 371–376.

Address of the authors:
DR. EDIT, TAKÁCS
DR. J. MARÓTI
Department of Botany, A. J. University,
H—6701 Szeged, P.O. Box 428.
Hungary

Acta Biol. Szeged. 30, pp. 75-89 (1984)

UPPER CRETACEOUS SPOROMORPHS FROM THE SOUTHERN PART OF HUNGARY (CSÁVOLY)

M. KEDVES

Department of Botany, Attila József University, Szeged

(Received December 7, 1982)

Abstract

The Senonian sediments of the bore-hole of Csávoly-I (South Hungary) were investigated palynologically. The spore-pollen composition is different from those described from the Bakony Region (Transdanubia). The presence of *Endoinfundibulapollis* TSCHUDY 1975 is also a pecularity of this assemblage, this form-genus has been reported previously. In our spore-pollen assemblage there are relatively high numbers of younger types, but at present it is best to give the geologic age, Upper Senonian, "Csávoly-type spore-pollen assemblage."

Key-words: Palynology, Upper Cretaceous, Hungary.

Introduction

Hungary is relatively rich in Senonian sedimentary deposits, but our palynologic knowledge is poor in relation to that of the other countries of Europe. Moreover, selected data have been published from only one region (Transdanubia) and there is no information presently available from other areas in Hungary.

The aim of the present investigation is a taxonomic elaboration of the complete spore-pollen assemblage of the south part of the Hungarian Plain, both from a paleoecological and stratigraphical point of view. This paper presents the first report on the Senonian palynology of South Hungary.

Materials and Methods

The investigation material was placed at my disposal by DR. M. MUCSI and DR. T. SZEDERKÉNYI (Department of Mineralogy, Geochemistry and Petrography, A. J. University, Szeged). The following samples were studied from the borehole Csávoly-I:

Csáv-I-1. — 18/1, 1271.5 — 1271.7 m., limestone
Csáv-I-2. — 19, 1310.5 — 1312.5 m., limestone
Csáv-I-3. — 23, 1487.0 — 1490.0 m., marl with aleurite
Csáv-I-4. — 24, 1540.0 — 1541.0 m., sandy marl
Csáv-I-5. — 25, 1550.0 — 1551.0 m., marl, with quartz and pyrit
Csáv-I-6. — 26, 1586.5 — 1587.5 m., marl, with sand and pyrit.

The samples were prepared by using HCl, washing — separation with $ZnCl_2$ — washing — HF — washing, and the slides were prepared using glycerin-jelly. Only the LM method was used during the course of this investigation but it seems that future electronmicroscopic studies would be of value projected.

Results

1. New taxa

Form-genus: *Romeinipollenites* Kds. and Herngr. 1980
Romeinipollenites hungaricus n. fsp.
(Plate III, figs. 9, 10, fig. 1)

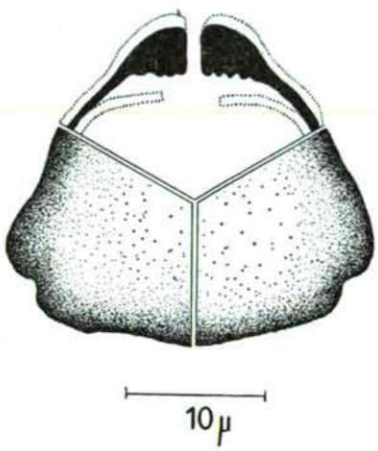


Fig. 1. *Romeinipollenites hungaricus* n. fsp.

Diagnosis

Equatorial contour triangular, with slightly convex sides. Surface smooth or very finely scabrate. Extragerminal exine is about 1–1.5 µm, the infratectal layer is the thinnest between the ectexine layers. The structure is not clearly definible using light microscope methods. Exoapertures consist of very short asymmetrical colpi. The annulus is 3–4 µm thick and the vestibulum is narrow and short. The diameter of the endopores is 4–5 µm.

Diameter: 24–30 µm.
Holotype: Plate III, figs. 9, 10, slide Csáv-I-1-6, 19.3/105.8.
Locus typicus: Csávoly.
Stratum typicum: limestone, upper Senonian.
Derivatio nominis: from Hungary.
Differential diagnosis: The smooth surface, the thinner annulus and the smaller size clearly separate this species from *R. granulatus* Kds. and Herngr. 1980.

Form-genus: *Interporopollenites* Weyl. and Krieg. 1953
Interporopollenites csavolyensis n. fsp.
(Plate IV, figs. 1, 2, fig. 2)

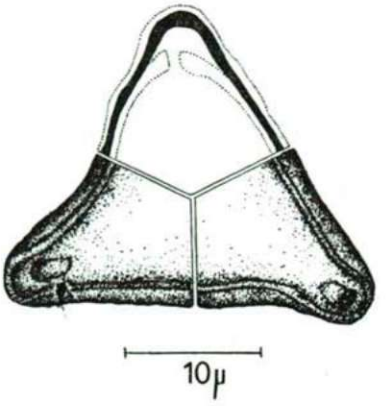


Fig. 2. *Interporopollenites csavolyensis* n. fsp.

Diagnosis

Amb triangular, with straight or slightly concave sides. Surface finely punctate-granulate. Extragerminal exine 1.5–1.8 μm thick, the tectum, infratectal layer and the foot layer are of equal thickness. The structure of the infratectal layer is not clearly visible by light microscopic methods, but it is probably granular. The apertural areas are connected by arcus-like thickenings. Exoapertures are radially elongated pores, 2×3 μm. Vestibulum 3 μm vide, endannulus 1.8 μm thick.

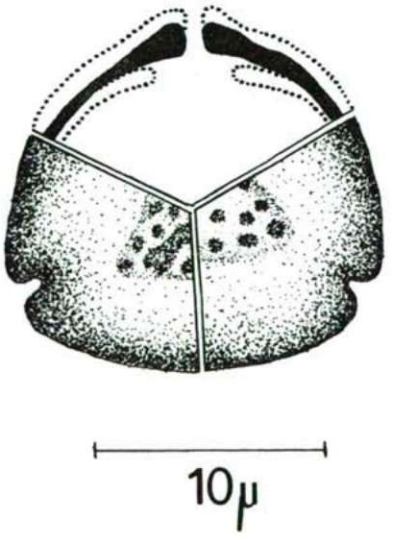


Fig. 3. *Papillopollis csavolyensis* n. fsp.

Diameter: 20–28 μm.
Holotype: Plate IV, figs. 1, 2, slide Csáv-I-2-3, 19.4/117.8.
Locus typicus: Csávoly.
Stratum typicum: limestone, upper Senonian.
Derivatio nominis: from Csávoly, the type locality.
Differential diagnosis: The smaller size and the punctate-granulate surface serve to distinguish *I. csavolyensis* from *I. plicatus* KDS. and HEG. 1975.

Form-genus: *Papillopollis* PF. 1953
Papillopollis csavolyensis n. fsp.
(Plate IV, figs. 23, 24, fig. 3)

Diagnosis

Amb triangular, with concave sides. Surface smooth or finely punctate. Interapertural exine is 2 μm thick, the foot layer is the thinnest between the ectexine layers. Structure of the infratectal layer is not clearly visible by light microscopy. Exoapertures are short asymmetrical colpi. Annulus is 2 μm thick, the infratectal layer is thickened in the apertural region. Endoapertures striate. On both polar areas there are papilli in the form of triangles. The papillus is atypical and consists of several tectum remnants which are usually circular but are sometimes irregular in outline.

Diameter: 14–20 μm.
Holotype: Plate IV, figs. 23, 24, slide Csáv-I-1-2, 17.3/110.3.
Locus typicus: Csávoly.
Stratum typicum: limestone, upper Senonian.
Derivatio nominis: from Csávoly, the type locality.
Differential diagnosis: The fine morphology of the polpapillus serves to distingish *P. csavolyensis* from *P. guinetii* KDS. and PITT. 1979 and the presence of an atrium separates it from *P. cretacicus* KDS. and PITT. 1979.

Papillopollis csavolyensis n. fsp. forma magna n. f.
(Plate IV, figs. 25, 26)
The specimens of this fsp. range in size from 21 to 28 μm in diameter.

2. The spore-pollen assemblage of the Senonian of Csávoly

2.1. Ecologically important sporomorphs: *Salviniaceae, Salvinia* v. *Azolla* (*Hydrosporis* ex gr. *levis* W. KR. 1962, pl. I, figs. 1. 2), *Gleicheniaceae* (*Dictyophyllidites* ex gr. *teuplitzensis* (W. KR. 1962) n. comb. *teuplitzensis*, pl. I, figs. 3, 4, syn: 1962, KRUTZSCH. — *Toroisporis* (*Toroisporis*) *teuplitzensis* n. fsp. subfsp. *teuplitzensis*, p. 78, pl. 32, figs. 1–10, *Dictyophyllidites* fsp. pl. I, figs. 5, 6), *Schizaeaceae, Schizaea* (*Cicatricososporites* fsp., pl. II, figs. 7, 8, *Reticulosporis* fsp., pl. II, figs. 9. 10), *Selaginellaceae* (*Echinatisporis* fsp., pl. I, figs. 15, 16), *Pteridaceae* (*Polypodiaceoisporites* fsp. 1, 2, pl. II, figs. 1, 2, 3, 4), *Polypodiaceae* (*Polypodiidites saalensis* (W. KR. 1959) W. KR. 1963, pl. II, figs. 5, 6), *Araucariaceae* (*Araucariacites balinkaense* KDS. 1974, pl. III. figs. 3,4).

2.2. The *Vadaszisporites* group is characteristic of the entire Cretaceous (Pl. I, figs. 7, 8, 9, 10), *Cristatitriletes* fsp. (pl. I, figs. 11, 12) and *Callisporites* fsp. (pl. I, figs. 13, 14) are relatively "old" spore types.

2.3. The very low number of gymnosperm pollen grains, and the lack of the *Classopollis* group also seems ecologically important. The few *Abietaceae* pollen grains (*Pityosporites* fsp., 1, 2, pl. II, figs. 11, 12, pl. III, figs. 1, 2) probably came from the highland vegetation.

2.4. From a paleophytogeographical point of view the *Endoinfundibulapollis* form-genus (*E. distinctus* R. TSCHUDY 1975, pl. IV, figs. 5, 6, E. fsp., pl. IV. figs. 7, 8) has a special importance.

2.5. Early *Angiosperm* pollen types, which first appear in the Lower Cretaceous: *Retitricolpites subtilimaculatus* LAING 1975, pl. VI, figs. 11, 12, *Retitricolpites exiguiexemplum* LAING 1975, pl. VI, figs. 13, 14.

2.6. Pollen types which have a general Senonian distribution: *Gemmamonocolpites* fsp., pl. III, figs. 5, 6, cf. *Longanulipollis* fsp., pl. III, figs. 7, 8, *Complexiopollis lusitanicus* KDS. and DIN. in print/a, pl. III, figs. 13, 14, *Interporopollenites zaklinskaiae* KDS. and HEG. 1975, pl. III, figs. 15, 16, *Interporopollenites csavolyensis* n. fsp., pl. IV, figs. 1, 2, *Interporopollenites stanleyi* KDS. and HEG. 1975, pl. IV. figs. 3, 4, *Suemegipollis triangularis* GÓCZ. 1964, pl. IV, figs. 13, 14, *Vacuopollis prezensis* KDS. and DIN. in print/a, pl. IV. figs. 15, 16, *Plicapollis silicatus* PF. 1953, pl. IV. figs. 17, 18, *Plicapollis serta* PF. 1953, pl. IV, figs. 19, 20, *Verruoculopollis* fsp., KDS. and DIN. in print/b, pl. IV, figs. 21, 22, *Papillopollis csavolyensis* n. fsp., pl. IV, figs. 23–26, *Trudopollis proparvus* PF. 1953, pl. V, figs. 3, 4, *Trudopollis hemiparvus* PF. 1953, pl. V, figs. 5, 6, *Trudopollis* fsp., pl. V, figs. 7, 8, cf. *Trudopollis* fsp., pl. V, figs. 9, 10, *Normapolles* fgen. et fsp. indet., pl. V, figs. 13, 14.

2.7. Maestrichtian — Danian Normapolles types: *Romeinipollenites hungaricus* n. fsp., pl. III, figs. 9, 10, *Magnoporopollis krutzschii* KDS. and HERNGR. 1980, pl. III, figs. 11, 12, *Pseudosculapollis tschudyi* KDS. and HERNGR. 1980, pl. IV, figs. 9, 10, *Elsikipollenites maastrichtiensis* KDS. and HERNGR. 1980, pl. IV, figs. 27, 28, *Trudopollis hojrupensis* KDS. 1979, pl. IV, figs. 29, 30, *Trudopollis triangulus* KDS. and HERNGR., pl. IV, figs. 31, 32, *Trudopollis lativerrucatus* KDS. and HERNGR. 1980, pl. V, figs. 1, 2, *Hofkeripollenites capsula* (PF. 1953) KDS. and HERNGR. 1980, pl. V, figs. 11, 12.

2.8. Cretaceous — Tertiary pollen grains, which are for the most part characteristic of the Lower Tertiary: *Rugutriporites balinkaense* (KDS. 1974) KDS. 1982 subfsp. *minor* KDS. 1974, cf. *Ulmaceae,* pl. V, fig. 15, 16, *Triporopollenites minimus* KDS. 1974, *Juglandaceae,* pl. V, figs. 17, 18, *Triporopollenites costatus* TAKAHASHI 1961, *Corylaceae,* pl. V, figs. 19, 20, *Subtriporopollenites constans* PF. 1953 subfsp. *medius* W. KR. and VANH. 1977, cf. *Juglandaceae,* pl. V, figs. 21, 22, *Subtriporopollenites constans* PF. 1953 cf. subfsp. *minor* KDS. 1970, pl. V, figs. 23, 24, *Subtriporopollenites facilis* (BOTSCH. 1960) KDS. 1970, *Juglandaceae,* pl. VI, figs. 1, 2, *Subtriporopollenites urkutensis* KDS. 1974, *Juglandaceae,* cf. CARYA, pl. VI, figs. 3, 4, cf. *Subtriporopollenites* fsp., pl. VI, figs. 5, 6, *Ulmoideipites krempii* ANDERS. 1960, *Ulmaceae,* pl. VI, figs. 7, 8, *Retitricolporites tenuiformis* (GROOT, PENNY, and GROOT 1961) KDS. in print, pl. VI, figs. 9, 10, *Cupuliferoipollenites oviformis* (R. POT. 1931) R. POT. 1960, *Fagaceae, Castanea,* pl. VI, figs. 15, 16, *Cyrillaceaepollenites exactus* (R. POT. 1931) R. POT. 1960, *Cyrillaceae, Clethraceae* v. *Theaceae,* pl. VI, figs. 17, 18, *Intragranulitricolporites microporus* KDS. 1978, pl. VI, figs. 19, 20, *Intragranulitricolporites wolffi* KDS. 1978, pl. VI, figs. 21, 22, *Intragranulitricolporites grambasti* KDS. 1978, pl. VI, figs. 23, 24, *Nagyipollis* fsp., pl. VI, fig. 25, 26.

Discussion and conclusions

1. From a paleophytogeographical point of view the first European occurrence of *Endoinfundibulapollis* TSCHUDY 1975 is noteworthy. This, and other facts (the lack of *Hungaropollis, Pseudopapillopollis*) clearly separate this assemblage from those of Transdanubia, previously described from the Bakony region. To better understand the palynostratigraphy of the Senonian layers of the Carpath Basin it is necessary to obtain palynological data from the Balkan Peninsule.

2. There are some problems regarding the palynological age of the Csávoly sedimentary deposits. It is pity that the palynological literature often includes reports only or exclusively of the stemma *Normapolles* as being present in assemblages from the Senonian. Therefore it is not so easy to establish the regional and stratigraphical value of the *Postnormapolles* and other taxa. The basic assemblage (Fish Clay, Lower Danian, KEDVES, 1979, Maestrichtian, stratotype, Gulpen Formation, KEDVES and HERNGREEN, 1980) are in the boreal region of the *Normapolles* province. Probably it will be necessary to create or modify the standards for the Mesogean region. In the Csávoly material there are pollen grains, in remarkable quantity, of so-called "younger type". *Pseudotrudopollis crassiexinus* is a pollen which seems to be characteristic of the Lower Maestrichtian. However, it is not present in the Csávoly section. Probably this is caused only by a regional difference. At present one could refer to it only as the "Upper Senonian, Csávoly-type".

3. The paleophytogeographical differences within the boreal and mesogean (Mediterranean) regions must be studied in detail. A review discussing this point of view was made by KEDVES and DINIZ (in print/b). Based on the conclusion presented in this paper, a knowledge of the complete spore-pollen assemblages is urgently needed from other localities, such as Italy and the Balkan Peninsule etc.

Acknowledgements

The writer is deeply indebted to DR. W. S. DRUGG (Chevron Oil Field Research Company, La Habra, California, U. S. A.) for critically reading the manuscript for linguistic errors.

Plate I

1, 2. *Hydrosporis* ex gr. *levis* W. KR. 1962, *Salvinia* v. AZOLLA, slide Csáv-I-2-6, 6.8/106.9.
3, 4. *Dictyophyllidites* ex gr. *teuplitzensis* (W. KR. 1962) n. comb. *teuplitzensis, Gleicheniaceae*, slide Csáv-I-1-3, 19.1/102.8.
5, 6. *Dictyophyllidites* fsp., *Gleicheniaceae*, slide Csáv-I-1-9, 14.7/116.4.
7, 8. *Vadaszisporites sacali* DEÁK and COMBAZ 1967, *Lycopodiaceae*, slide Csáv-I-2-3, 20.0/104.6.
9, 10. Cf. *Vadaszisporites* fsp., slide Csáv-I-1-2: 6.9/118.0.
11, 12. *Cristatitriletes* fsp., slide Csáv-I-2-2, 10.8/107.8.
13, 14. *Callisporites* fsp., slide Csáv-I-3-5, 3.9/113.8.
15, 16. *Echinatisporis* fsp., *Selaginellaceae*, slide Csáv-I-1-5, 7.2/118.9.
N: x1000

Plate II

1, 2. *Polypodiaceoisporites* fsp.$_1$, *Pteridaceae*, slide Csáv-I-1-10, 19.7/101.4.
3, 4. *Polypodiaceoisporites* fsp.$_2$, *Pteridaceae*, slide Csáv-I-6-3, 19.7/119.0.
5, 6. *Polypodiidites senalensis* (W. KR. 1959) W. KR. 1963, *Polypodiaceae*, slide Csáv-I-1-4, 14.9/104.3.
7, 8. *Cicatricosoporites* fsp., *Schizaeaceae, Schizaea*, slide Csáv-I-3-7, 13.5/106.9.
9, 10. *Reticulosporis* fsp., *Schizaeaceae, Schizaea*, slide Csáv-I-2-7, 16.7/110.3.
11, 12. *Pityosporites* fsp.$_1$, *Abietaceae*, slide Csáv-I-1-2, 7.8/105.1.
N: x1000

Plate III

1, 2. *Pityosporites* fsp.$_2$, *Abietaceae*, slide Csáv-I-1-1, 10.3/117.8.
3, 4. *Araucariacites balinkaense* KDs. 1974, *Araucariaceae*, slide Csáv-I-1-2, 15.9/116.6.
5, 6. *Gemmamonocolpites* fsp., slide Csáv-I-3-5, 16.6/103.6.
7, 8. Cf. *Longanulipollis* fsp., slide Csáv-I-1-1, 16.3/110.9.
9, 10. *Romeinipollenites hungaricus* n. fsp., slide Csáv-I-1-6, 19.3/105.8.
11, 12. *Magnoporopollis krutzschii* KDs. and HERNGR. 1980, slide Csáv-I-2-1, 5.3/104.9.
13, 14. *Complexiopollis lusitanicus* KDs. and DIN. in print/a, slide Csáv-I-1-3, 10.7/105.4.
15 16. *Interporopollenites zaklinskaiae* KDs. and HEG. 1975, slide Csáv-I-1-2, 12.8/110.3.
N: x1000

Plate IV

1, 2. *Interporopollenites csavolyensis* n. fsp., slide Csáv-I-2-3, 19.4/117.8.
3, 4. *Interporopollenites stanleyi* KDs. and HEG. 1975, slide Csáv-I-1-8, 19.9/114.1.
5, 6. *Endoinfundibulapollis distinctus* R. TSCHUDY 1975, slide Csáv-I-2-6, 17.6/111.7.
7, 8. *Endoinfundibulapollis* fsp., slide Csáv-I-1-3, 12.9/107.3.
9, 10. *Pseudosculapollis tschudyi* KDs. and HERNGR. 1980, slide Csáv-I-1-1, 15.2/114.8.
11, 12. *Pseudosculapollis* fsp., slide Csáv-I-1-4, 15.8/108.3.
13, 14. *Suemegipollis triangularis* GÓCZ. 1964, slide Csáv-I-2-9, 14.6/104.7.
15, 16. *Vacuopollis prezensis* KDs. and DIN. in print/a, slide Csáv-I-1-8, 19.6/117.8.
17, 18. *Plicapollis silicatus* PF. 1953, slide Csáv-I-1-5, 7.4/117.5.
19, 20. *Plicapollis serta* PF. 1953, slide Csáv-I-1-9, 20.8/109.5.
21, 22. *Verruoculopollis* fsp., KDs. and DIN. in print/b, slide Csáv-I-1-10, 12.7/109.5.
23, 24. *Papillopollis csavolyensis* n. fsp., subfsp. *csavolyensis*, slide Csáv-I-1-2, 17.3/110.3.
25, 26. *Papillopollis csavolyensis* n. fsp. forma *magna* n. f., slide Csáv-I-1-5, 17.4/109.3.
27, 28. *Elsikipollenites maastrichtiensis* KDs. and HERNGR. 1980, slide Csáv-I-1-2, 21.1/113.7.
29, 30. *Trudopollis hojrupensis* KDs. 1979, slide Csáv-I-1-8, 17.3/116.8.
31, 32. *Trudopollis triangulus* KDs. and HERNGR. 1980, slide Csáv-I-1-2, 15.5/111.9.
N: x1000

Plate V

1, 2. *Trudopollis lativerrucatus* KDs. and HERNGR. 1980, slide Csáv-I-1-9, 16.1/115.3.
3, 4. *Trudopollis proparvus* PF. 1953, slide Csáv-I-1-6, 8.6/116.8.
5, 6. *Trudopollis hemiparvus* PF. 1953, slide Csáv-I-1-1, 8.3/114.7.
7, 8. *Trudopollis* fsp., slide Csáv-I-1-3, 14.7/116.9.
9, 10. Cf. *Trudopollis* fsp., slide Csáv-I-1-6, 12.9/116.2.
11, 12. *Hofkeripollis capsula* (PF. 1953) KDs. and HERNGR. 1980, slide Csáv-I-1-2, 18.3/105.4.
13, 14. *Normapolles* fgen. et fsp. indet., slide Csáv-I-1-1, 8.2/106.3.
15, 16. *Rugutriporites balinkaense* (KDs. 1974) KDs. 1982 subfsp. *minor* KDs. 1974, cf. *Ulmaceae*, slide Csáv-I-1-10, 6.7/110.8.
17, 18. *Triporopollenites minimus* KDs. 1974, cf. *Juglandaceae*, slide Csáv-I-1-4, 4.6/103.3.
19, 20. *Triporopollenites costatus* TAKAHASHI 1961, *Corylaceae*, slide Csáv-I-1-5, 16.8/105.3.
21, 22. *Subtriporopollenites constans* PF. 1953 subfsp. *medius* W. KR. and WANH. 1977, cf. *Juglandaceae*, slide Csáv-I-1-8, 8.5/105.8.
23, 24. *Subtriporopollenites constans* PF. 1953 cf. subfsp. *minor* KDs. 1970, slide Csáv-I-1-9, 13.6/115.9
N: x1000

Plate VI

1, 2. *Subtriporopollenites facilis* (BOTSCH. 1960) KDs. 1970, *Juglandaceae*, slide Csáv-I-1-7, 9.5/112,4.
3, 4. *Subtriporopollenites urkutensis* KDs. 1974, *Juglandaceae*, cf. *Carya*, slide Csáv-I-1-1, 16.1/117.0.
5, 6. Cf. *Subtriporopollenites* fsp., slide Csáv-I-1-1, 12.2/114.4.
7, 8. *Ulmoideipites krempii* ANDERS. 1960, *Ulmaceae*, slide Csáv-I-1-1, 20.3/115.2.
9, 10. *Retitricolpites tenuiformis* (GROOT, PENNY and GROOT, 1961) KDs. in print, slide Csáv-I-1-2, 9.7/108.5.
11, 12. *Retitricolpites subtilimaculatus* LAING 1975, slide Csáv-I-1-2, 11.4/110.6.

13, 14. *Retitricolpites exiguiexemplum* LAING 1975, slide Csáv-I-1-1, 21.2/110.9.
15, 16. *Cupuliferoipollenites oviformis* (R. POT. 1931) R. POT. 1960, *Fagaceae, Castanea*, slide Csáv-I-1-1, 15.0/109.8.
17, 18. *Cyrillaceaepollenites exactus* (R. POT. 1931) R. POT. 1960, *Cyrillaceae, Clethraceae* v. *Theaceae*, slide Csáv-I-1-1, 6.1/118.7.
19, 20. *Intragranulitricolporites microporus* KDS. 1978, slide Csáv-I-1-9, 15.7/102.4.
21, 22. *Intragranulitricolporites wolffi* KDS. 1978, slide Csáv-I-1-1, 19.0/106.0.
23, 24. *Intragranulitricolporites grcmbasti* KDS. 1978, *Fabaceae*, slide Csáv-I-1-2, 16.7/116.9.
25, 26. *Nagyipollis* fsp., slide Csáv-I-2-4, 18.6/107.0.
N: x1000

Plate I.

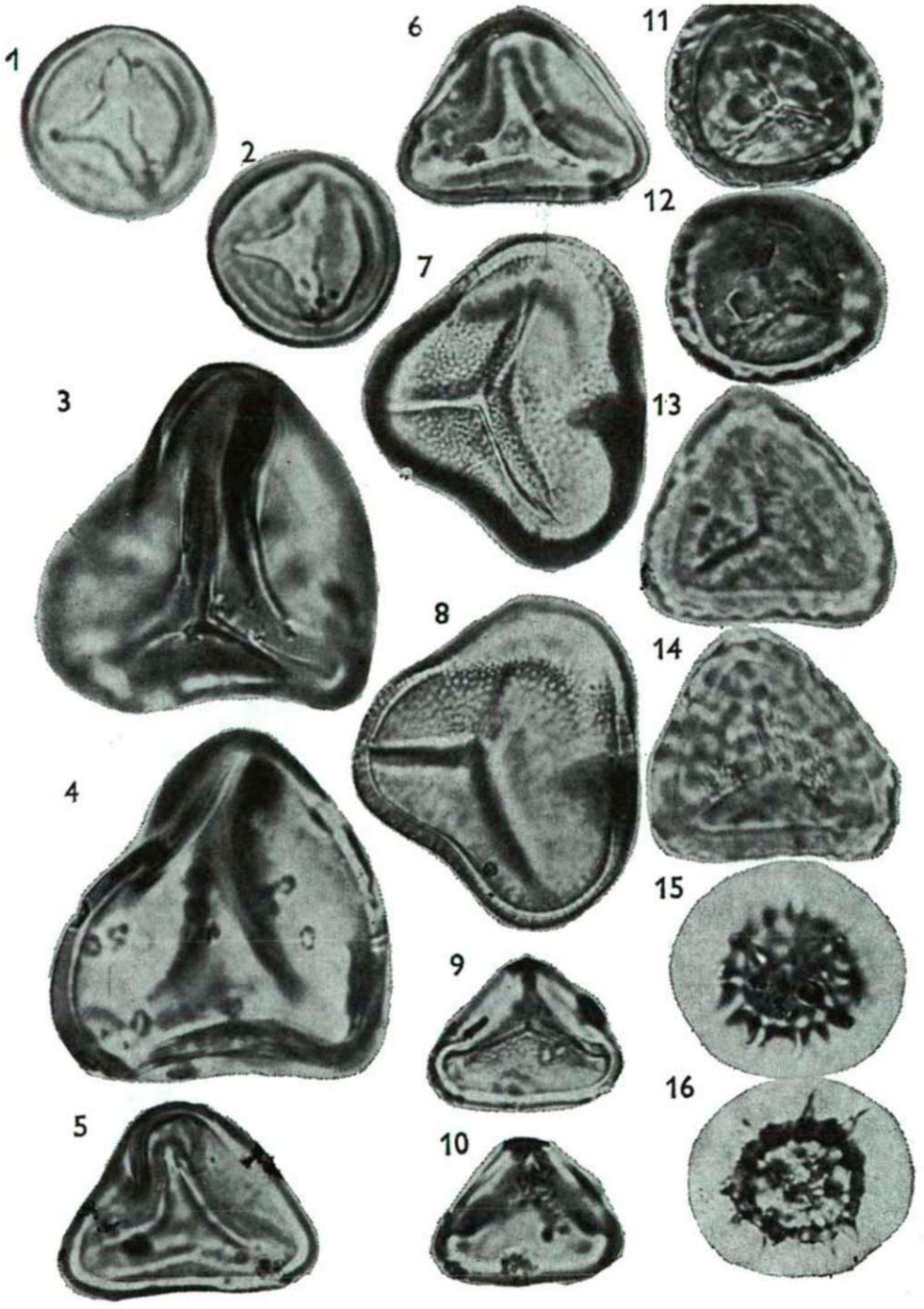


Plate II.

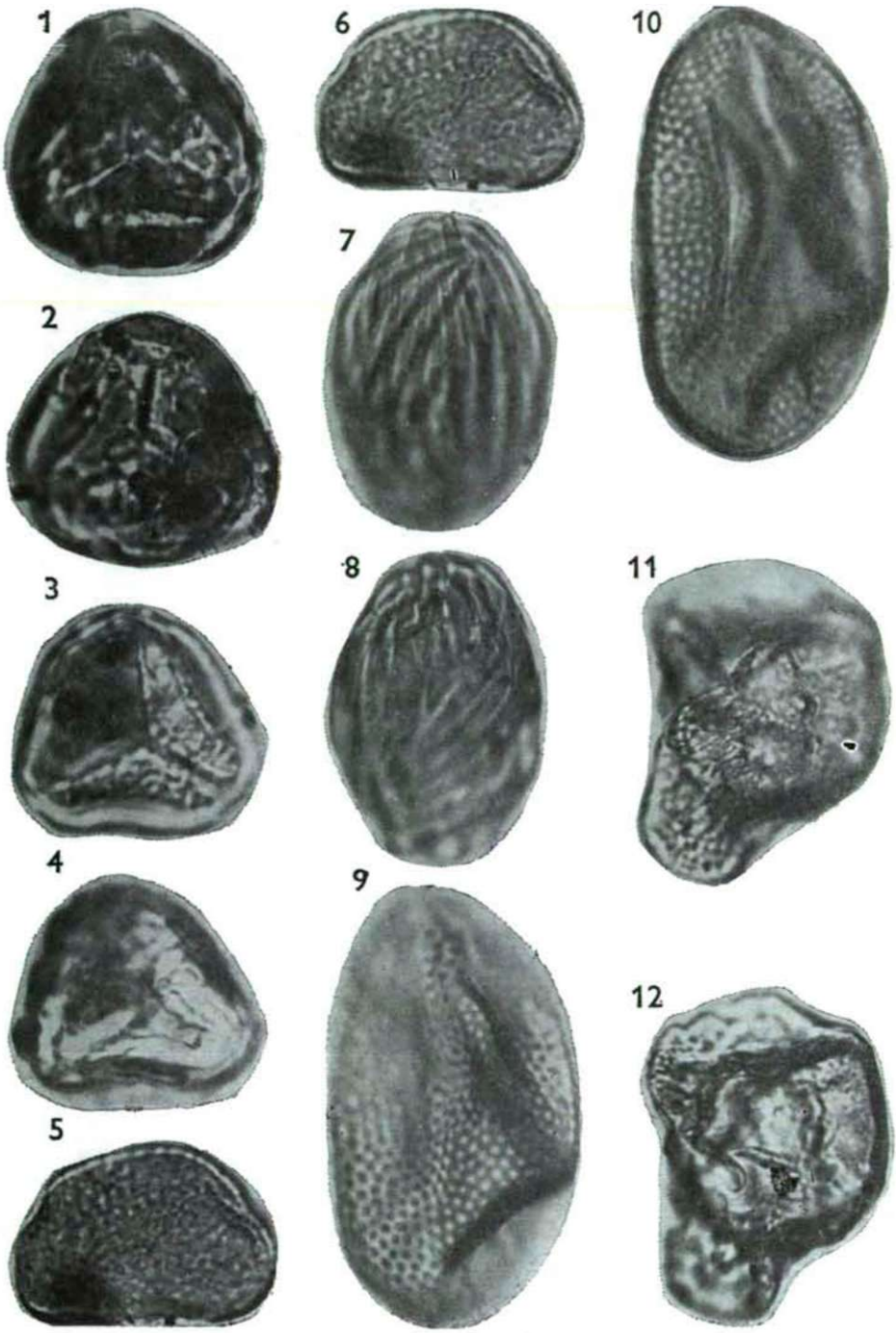


Plate III.

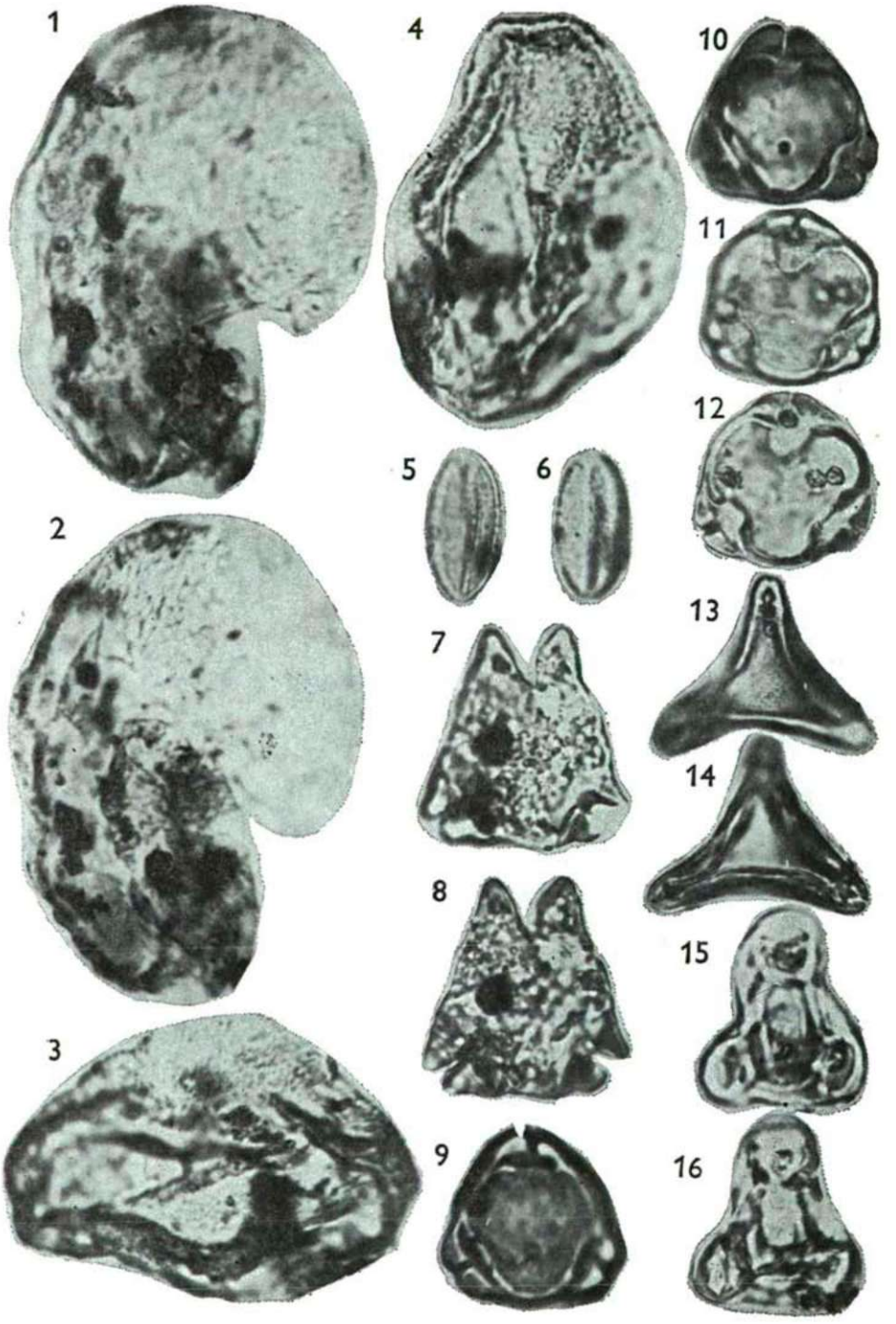


Plate IV.

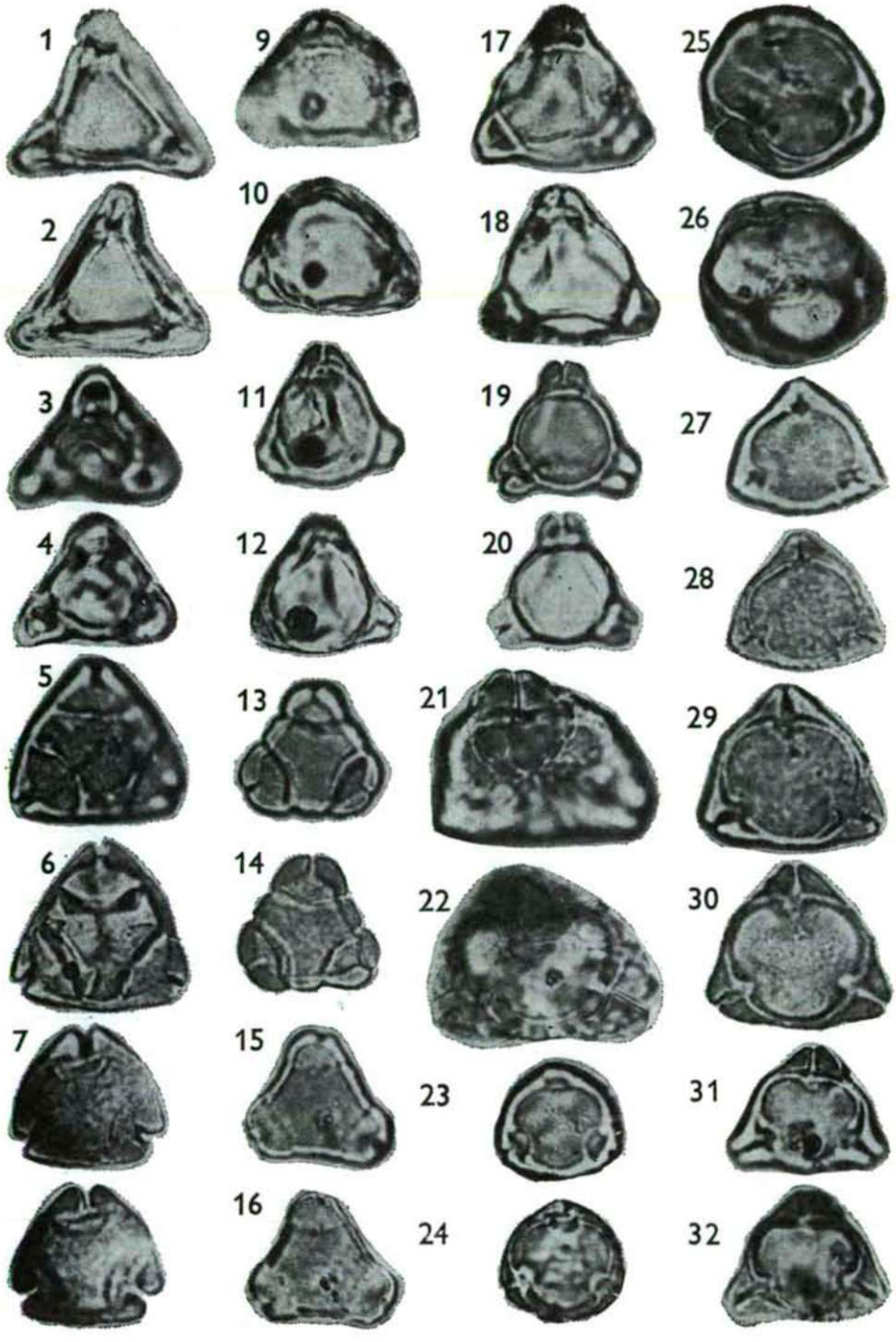

1
2
3
4
5
6
7
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32

Plate V.

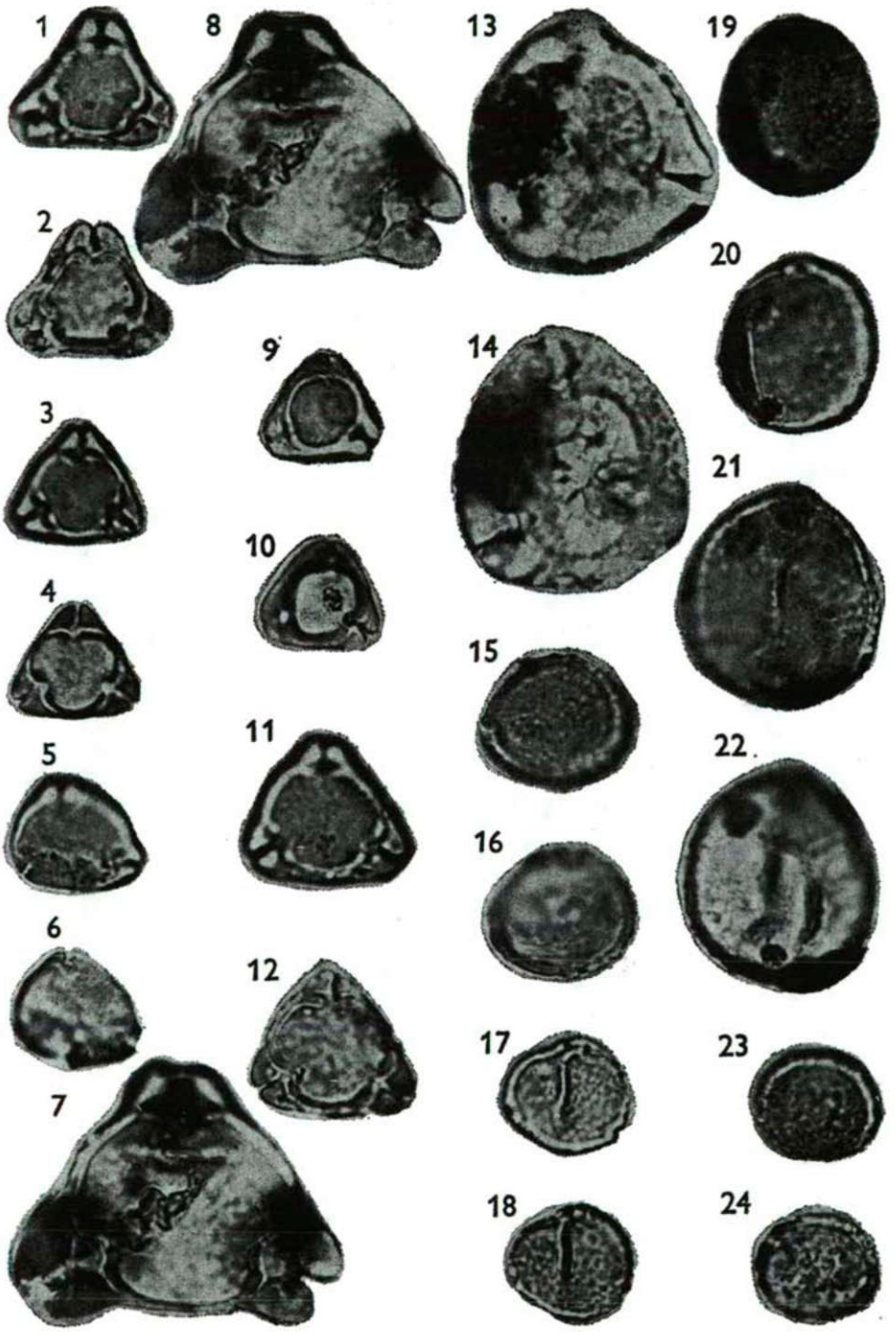
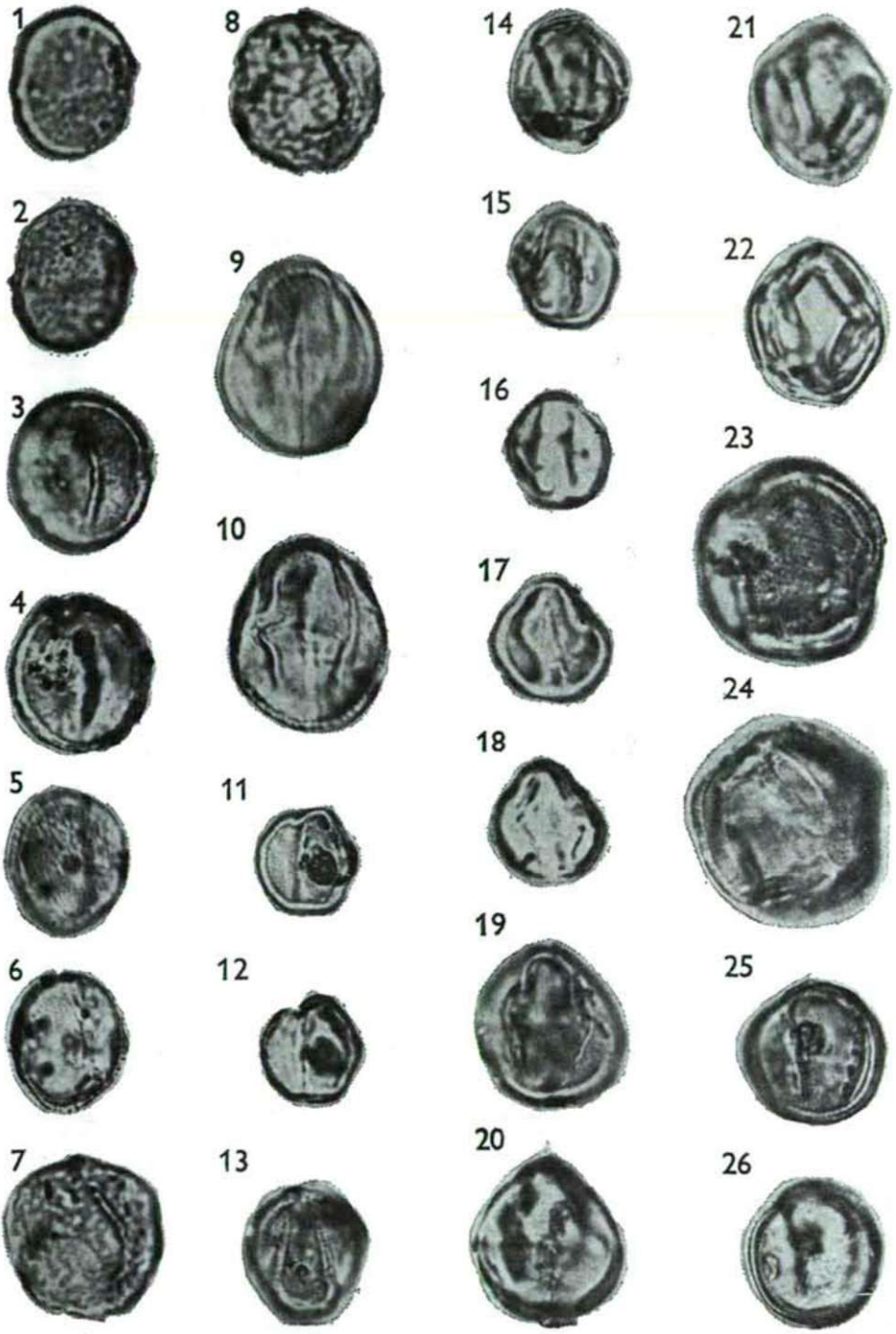


Plate VI.



References

ANDERSON, R. Y. (1960): Cretaceous-Tertiary Palynology, Eastern Side of the San Juan Basin, New Mexico. — N. Mex. Bureau of Mines *6*, 1–59.

DEÁK, H. M. and COMBAZ, A. (1967): "Microfossiles organiques" du Wealdien et du Cénomanien dans un sondage de Charente-Maritime. — Rev. de Micropaléontologie *10*, 69–96.

GÓCZÁN, F. (1964): Stratigraphic Palynology of the Hungarian Upper Cretaceous. — Acta Geologica *8*, 229–264.

GROOT, J. J., PENNY, J. S. and GROOT, C. R. (1961): Plant microfossils and age of the Raritan, Tuscaloosa and Magothy Formations of the Eastern United States. — Palaeontographica B, *108*, 121–140.

KEDVES, M. (1970): Etudes palynologiques des couches du Tertiaire inférieur de la Région Parisienne. V. Pollens triporés, subtriporés et intratriporés. — Pollen et Spores *12*, 83–97.

KEDVES, M. (1974): Paleogene fossil sporomorphs of the Bakony Mountains. Part II. — Studia Biol. Hung. *13*, 1–124.

KEDVES, M. (1978): Paleogene fossil sporomorphs of the Bakony Mountains. Part III. — Studia Biol. Hung. *15*, 1–166.

KEDVES, M. (1979): Palynological investigations on sediments of the Lower Danian (Fish Clay, Denmark). I. — Acta Miner. — Petr. Szeged *24*, 167–186.

KEDVES, M. (1980): Palynological investigations on sediments of the Lower Danian (Fish Clay, Denmark). II. Acta Miner. — Petr. Szeged *24*, 355–376.

KEDVES, M. and DINIZ, F. (in print a): Contribution à la connaissance des pollens d'angiospermes du Crétacé supérieur de Portugal.

KEDVES, M. and DINIZ, F. (in print b): Nouvelles espèces de Normapolles du Crétacé supérieur mésogéen. Contribution paléobiogeographique.

KEDVES, M. and HEGEDŰS, M. (1975): Pollen grains of the Interporopollenites fgen. from sediments of the Upper Cretaceous period in Portugal. — Acta Biol. Szeged. *21*, 43–62.

KEDVES, M. and HERNGREEN, G. F. W. (1980): Palynology of the stratotype of the Maestrichtian and the Gulpen Formation, ENCI Section, Maastricht, The Netherlands. — Pollen et Spores *22*, 483–544.

KEDVES, M. and PITTAU, P. (1979): Contribution à la connaissance des pollens des Normapolles du "groupe papilloide" du Crétacé supérieur du Portugal. — Pollen et Spores *21*, 169–209.

KEDVES, M. and RUSSELL, D. E. (1982): Palynology of the Thanetian layers of Menat. — Palaeontographica B, *182*, 87–150.

KRUTZSCH, W. (1959): Mikropaläontologische (sporenpaläontologische) Untersuchungen in der Braunkohle des Geiseltales. — Geologie *8*, BH 21/22, 1–425.

KRUTZSCH, W. (1962): Atlas der mittel- und jungtertiären dispersen Sporen- und Pollen- sowie der Mikroplanktonformen des nördlichen Mitteleuropas. — Lief. I. Laevigate und toriate trilete Sporenformen. — VEB Deutscher Verlag der Wissenschaften, Berlin.

KRUTZSCH, W. (1963): Beitrag zur Kenntnis der Mikroflora der Niederschöner Schichten: Eine kleine Mikroflora aus der Bohrung Königstein. — Berichte der Geol. Ges. *8*, 224–236.

LAING, J. F. (1975): Mid-Cretaceous angiosperm pollen from southern England and northern France. — Palaeontology *18*, 775–808.

PFLUG, H. D. (1953): Zur Entstehung und Entwicklung des angiospermiden Pollens in der Erdgeschichte. — Palaeontographica B, *95*, 60–171.

POTONIÉ, R. (1931): Zur Mikroskopie der Braunkohlen. Tertiaere Bluetenstaubformen, (1. Mitt.). — Z. Braunkohle *16*, 325–333.

POTONIÉ, R. (1960): Synopsis der Gattungen der Sporae dispersae III. Teil: Nachträge Sporites, Fortsetzung Pollenites. Mit Generalregister zu Teil. I–III. — Beih. Geol. Jb. *39*, 1–189.

TAKAHASHI, K. (1961): Pollen und Sporen des westjapanischen Alttertiärs und Miozäns. — Mem. Fac. Sci. Kyushu Univ. D, *11*, 279–345.

THOMSON, P. W. and PFLUG, H. D. (1953): Pollen und Sporen des mitteleuropäischen Tertiärs. — Palaeontographica B, *94*, 1–138.

TSCHUDY, R. H. (1975): Normapolles pollen from the Mississippi embayment. — U.S. Geol. Surv. Prof. Pap. *865*, 1–42.

WEYLAND, H. and KRIEGER, W. (1953): Die Sporen und Pollen der Aachener Kreide und ihre Bedeutung für die Characterisierung des Mittleren Senons. — Palaeontographica B, *95*, 6–29.

Address of the author:
DR. M. KEDVES
Department of Botany, A. J. University,
H-6701 Szeged, P.O. Box 428
Hungary

Acta Biol. Szeged. 30, pp. 91–105 (1984)

ÉTUDE PALYNOLOGIQUE D'UN LIGNITE TERTIAIRE DE BLAO, VIET-NAM -I-

M. KEDVES

Institut botanique de l'Université Attila József de Szeged, Hongrie

(Reçu, le 5 janvier 1983)

Sommaire

L'étude du contenu sporo-pollinique d'un échantillon de lignite provenant de la région de Blao (Sud du Viet-Nam) permet de mettre en évidence 12 espèces de forme de spores, 7 espèces de pollens de Gymnospermes, et 56 espèces de pollens d'Angiospermes. Parmi celles-ci deux genres et 50 espèces de forme sont nouveaux. La composition pollinique indique une végétation tropicale. Quelques formes; p. ex. *Dacrydiumites* sont probablement originaires d'Australie. La forme dominante est un pollen de Palmiers affine au genre *Didymosperma;* l'abondance du genre *Ilex* est également remarquable. Le pollen d'Ilex est connu en grande quantité dans les couches Pliocènes de Japon. Au point de vue de l'âge, l'absence de pollen de Composés et d'Ombellifères pose un problème. Nous pouvons fixer l'âge probable au Pliocène, mais le Miocène n'est pas tout à fait exclu.

Mots clés: Palynologie — Tertiaire supérieur — Viet-Nam.

Introduction

La matière d'investigation, un échantillon provenant des sédiments houilliers du Blao, a été prélevé par le Professeur SAURIN en Juin 1965 et mis à ma disposition par MONS. PH. GUINET, directeur adjoint du Laboratoire de Palynologie, Montpellier. De plus, MONS. PH. GUINET m'a communiqué des renseignements bibliographiques importants concernant la végétation actuelle du Sud du Viet-Nam. Je tiens à leur exprimer mes remerciements les plus sincères pour leur aide cordiale. L'âge des sédiments houilliers, selon les communications antérieures n'est pas fixé; Pliocène?

Étant donné, que, à notre connaissance il n'existe aucun document concernant la Palynologie du Viet-Nam, toutes données sur ce sujet ont une importance primordiale. Nous espérons, que plusieurs travaux suivront cette étude.

Matière et methode

L'echantillon a été préparé par l'attaque à l'acide nitrique (DELCOURT, MULLENDERS et PIÉRAT 1959) puis les bitumes ont été éliminés par l'action de KOH. En dernier lieu, on a traité par FH. Les études au microscope optique ont été effectuées par un microscope NFPK avec l'immersion à huile x100. Les microphotos ont été faits à un grossissement de x1000, mais les planches ont été réduites selon la justification de la revue.

Résultats

Note. — Locus typicus: Blao, stratum typicum: couches houillières.

Fgen.: *Baculatisporites* Pf. et Th. 1953.

La révision des spores fossiles des *Osmundaceae* du Tertiaire a été effectuée par Krutzsch (1967).

1. *Baculatisporites blaoensis* n. fsp. (Planche I, fig. 1, 2).

Diagnose

Contour équatorial circulaire ou elliptique. Les branches de la marque de tétrade n'atteignent pas le contour équatorial, r = 1/2–3/4. L'épaisseur de l'exospore de 1 à 1,7 μm. L'exospore se compose de deux couches, suivant les études au microscope optique. La couche extérieure est beaucoup plus épaisse que la couche intérieure. L'ornementation est très variable, baculée, ces éléments sont larges; quelquefois leurs extrémités sont épaissiès, la sculpture est alors clavée. La plus grande partie de l'ornementation est verruqueuse ou rugulée, la base des éléments de 2 à 4 μm. Autour des laesurae les éléments sculpturaux forment une pseudotorus.

Plus grande dimension: 55–70 μm.

Holotype: Planche I, fig. 1, 2, prep. Blao-17; 15, 6 /112, 4.

Derivatio nominis: de la localité type.

Diagnose différentielle: la sculpture du *B. quintus* (Pf. et Th. 1953) W. Kr. 1967 est de forme polygonale ou allongée. Les éléments de la sculpture sont papilloides, baculés ou verruqueux chez le *B. primarius* (Wolff 1934) Th. et Pf. 1953. Ces caractères sont bien distincts de ceux de l'espèce de forme nouvelle décrite ci-dessous. Il y a lieu de noter que la différence entre les espèces mentionnées plus haut n'est pas très grande.

Appartenance botanique probable: *Osmundaceae, Osmunda; O. presliana* Sm. type selon Krutzsch (1967) de Hongkong.

Fgen.: *Foveotriletes* Van der Hammen 1954 ex R. Pot. 1956.

1. *Foveotriletes asiaticus* n. fsp. (Planche I, fig. 3, 4).

Diagnose

Contour équatorial sub-triangulaire convexe, à angles largement arrondis. Les laesurae de la marque de tétrade n'atteignent pas le contour équatorial, r = 3/4–4/5. Autour des laesurae il y a des bourrelets arrondis minces, de 1,5 μm d'épaisseur. Ces bourrelets sont prominents et semblables à ceux du genre de forme *Biretisporites*. L'épaisseur de l'exospore est de 1,5 à 2 μm; elle se compose de deux couches, la couche inférieure est beaucoup plus épaisse que la couche extérieure. Le diamètre des perforations n'atteint pas en général 0,5 μm; elles sont irrégulièrement disposées.

Plus grande dimension: 48–60 μm.

Holotype: Planche I, fig. 3, 4, prep. Blao-7; 19,0/107,2.

Derivatio nominis: de l'Asie.

Diagnose différentielle: la dimension et le contour distinguent bien l'espèce de forme nouvelle de celles décrites par Krutzsch (1967). Krutzsch (1962) a décrit le fgen. *Microfoveolatisporis* qu'il distingue essentiellement du genre *Foveotriletes* par le contour. Ici, il y a lieu de souligner, que ce ne peut pas être un caractère distinctif; d'autre part il est intéressant que les spores du Blao sont de ce point de vue entre les deux genres mentionnés plus haut. *F. margaritae* (Van der Hammen 1954) Germ. et al., 1968 est plus petit et le diametre des perforations (foveolae) de 0,5 à 2 μm par rapport de notre espèce nouvelle.

Appartenance botanique probable: *Filicinae.*

2. *Foveotriletes hammenii* n. fsp. (Planche I, fig. 5, 6).

Diagnose

Contour équatorial triangulaire convexe, coins largement arrondis. Les laesurae, atteignent en général le contour équatorial et souvent se divisent à leurs extrémités. L'exospore est épaisse de 1 µm environ, elle se compose de deux couches, la couche extérieure est plus épaisse que la couche intérieure. Les perforations, de 0,5 à 0,7 µm de diamètre, sont quelquefois de forme allonguée de 2 à 3,5 µm de longueur.

Plus grande dimension: 32–40 µm.

Holotype: Planche I, fig. 5, 6, prep. Blao-12; 5,1/117,5.

Derivatio nominis: en hommage au Professeur T. VAN DER HAMMEN (Amsterdam) qui a décrit le premier ce genre de forme.

Diagnose différentielle: la taille plus petite, les laesures plus longues et les perforations parfois allongés distinguent nettement cette espèce du *F. asiaticus* n. fsp.

Appartenance botanique probable: *Filicinae.*

Fgen.: *Polypodiaceoisporites* R. POT. 1956 non 1951

1. *Polypodiaceoisporites saurinii* n. fsp. (Planche I, fig. 7, 8).

Diagnose

Contour équatorial triangulaire, côtés nettement concaves ou convexes, angles arrondis. Zone large de 3 à 4 µm environ. Les laesurae sont longues, mais n'atteignent pas toujours le bord interne de la zone, r = 4/5–5/5. Autour des laesurae il y a un bourrelet arrondi de 2 à 3 µm de largeur. La sculpture de la surface proximal du corps central est granuleuse à verruqueuse, ces éléments variant de 0,7 à 1,5 µm de diamètre. Les verrues s'anastomosent souvent et la sculpture est parfois rugulée. La longueur de ces éléments est variable, en général de 3 à 5 µm, la largueur est 1,5 µm environ. La sculpture de la face distale est presque identique à celle de la surface proximale, seule l'ornementation rugulée y est plus fréquente.

Plus grande dimension: 35–45 µm.

Holotype: Planche I, fig. 7, 8, prep. Blao-13; 8,6/107,2.

Derivatio nominis: en hommage à Mons. le Professeur SAURIN, qui a prélevé l'échantillon étudié.

Diagnose différentielle: le *P. verruspeciosus* W. KR. 1959 est plus petit et la sculpture du corps central est plus frappante que chez l'espèce de forme nouvelle décrite. La sculpture verruqueuse caractéristique du corps central du *P. saxonius* W. KR. 1967 est un caractère distinctif. La sculpture rugulée mesure de 2 à 4 µm du *P. rugulatearis* KDS. et J. R. 1965. Le *P. brevisculptatus* KDS. 1973 est beaucoup plus petite que l'espèce de forme décrite.

Appartenance botanique probable: *Pteridaceae.*

2. *Polypodiaceoisporites guinetii* n. fsp. (Planche I, fig. 9, 10).

Diagnose

Contour équatorial triangulaire, côtés nettement concaves ou convexes, angles largement arrondis. Zone large de 3 à 5 µm. Les laesurae sont longues mais n'atteignent pas en général le bord interne de la zone. Les laesurae sont nettement sinueuses. Elles sont entourées par un torus remarquable, de 2 à 3 µm de longueur. L'ornementation de la surface proximale du corps central est fine, verruqueuse-rugulée. Le diamètre, c'est dire la largeur des éléments de la sculpture est 1 µm environ. La sculpture de la surface distale est orientée selon la direction des rayons de la spore. Cette ornementation est complétement différente à celle de la surface proximale qui

est rugulée; la dimension des éléments de la sculpture est de 3 à 6 µm. Rarement il y a des verrues de 2 µm de diamètre.

Plus grande dimension: 40–50 µm.

Holotype: Planche I, fig. 9, 10, prep. Blao-7; 21,1/110,0.

Derivatio nominis: en hommage à MONS. PH. GUINET directeur adjoint à l'E. P. H. E. Laboratoire de Palynologie, Montpellier.

Diagnose différentielle: le *P. triangulus* KDS. et J. R. 1965 est beaucoup plus petite, et l'ornementation de l'hemisphère distal est de forme de triangle. La sculpture proximale du *P. tatabányensis* KDS. 1966 est complétement différente que notre espèce de forme nouvelle.

Appartenance botanique probable: *Pteridaceae.*

Fgen.: *Undulozonosporites* KDS. 1966.

1. *Undulozonosporites blaoensis* n. fsp. (Planche I, fig. 11, 12).

Diagnose

Contour équatorial triangulaire à angles largement arrondis. Côtés nettement convexes ou concaves. Une zone de 1 à 3 µm large est nettement ondulée. Les laesurae de la marque de tétrade sont longues, mais en général n'atteignent pas le bord interne de la zone, r = 4/5. Autour des laesurae il y a un torus de 2 à 3 µm de large. La sculpture de la surface proximale du corps central est finement rugulée, parfois verruqueuse, les éléments de la sculpture mesurent de 1,5 à 2 µm. Le caractère de l'ornementation de la surface distale du corps central est identique avec celle de la surface proximale, mais les éléments sont plus grands et plus allongés, de 2 à 2,5 µm environ.

Plus grande dimension: 35–45 µm.

Holotype: Planche I, fig. 11, 12, prep. Blao-13; 19,9/104,1.

Derivatio nominis: de la localité type.

Diagnose différentielle: il y a une seule forme semblable (fsp. 2, KEDVES, 1973) dans la litérature, les autres espèces de forme Européennes sont complétement différentes.

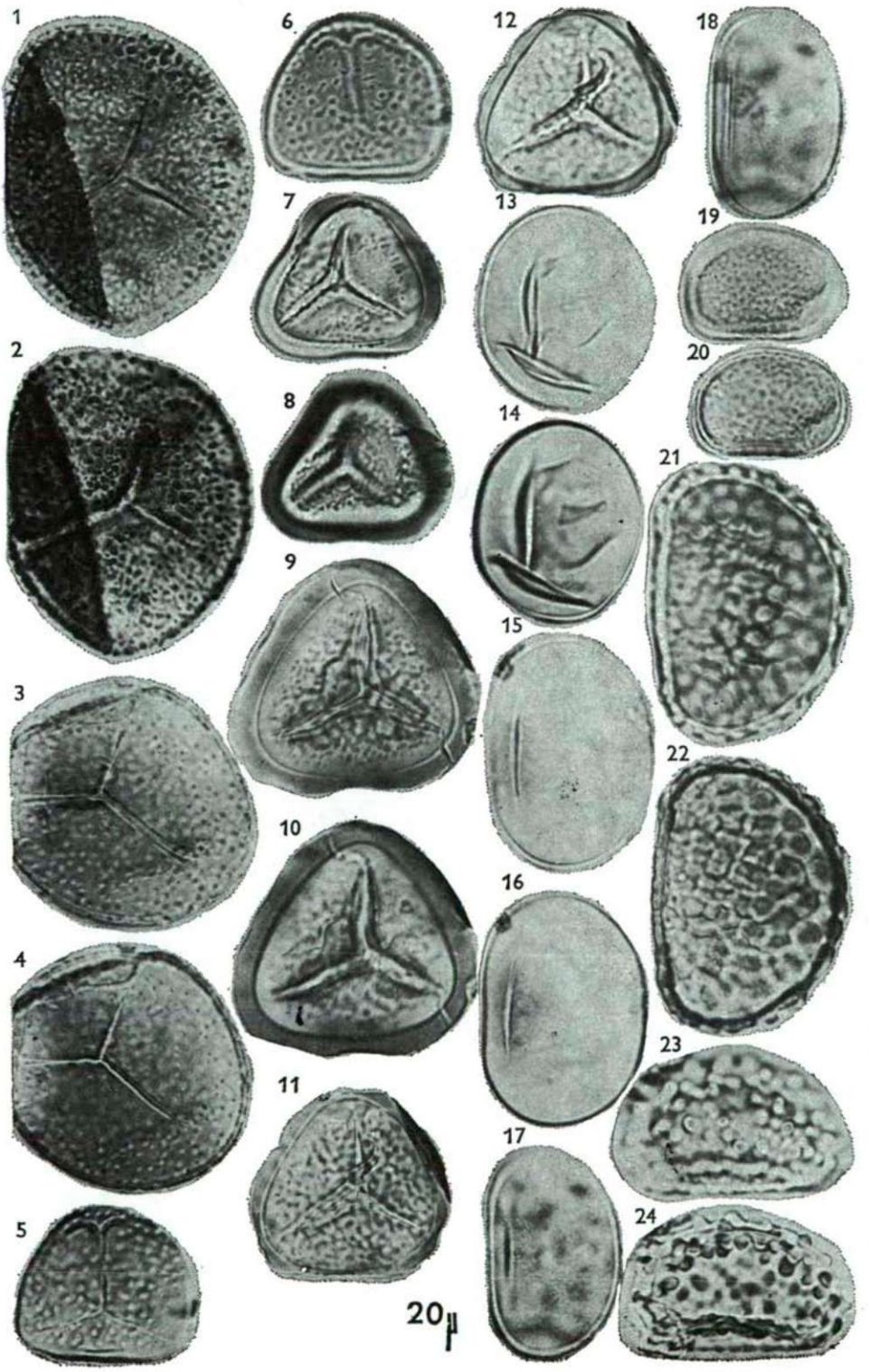
Appartenance botanique probable: *Pteridaceae.*

Fgen.: *Laevigatosporites* IBR. 1933.

1. *Laevigatosporites haardti* (R. POT. et VEN. 1934) TH. et PF. 1953 subfsp. *haardtioides* W. KR. 1967, *Polypodiaceae* (Planche I, fig. 13, 14).

Légende de la Planche I

Fig. 1, 2. *Baculatisporites blaoensis* n. fsp., *Osmundaceae, Osmunda,* prep. Blao-17; 15.6/112.4.
Fig. 3, 4. *Foveotriletes asiaticus* n. fsp., prep. Blao-7; 19.0/107.2.
Fig. 5, 6. *Foveotriletes hammenii* n. fsp., prep. Blao-12; 5.1/117.5.
Fig. 7, 8. *Polypodiaceoisporites saurinii* n. fsp., *Pteridaceae,* prep. Blao-13; 8.6/107.2.
Fig. 9, 10. *Polypodiaceoisporites guinetii* n. fsp., Pteridaceae, prep. Blao-7; 21.1/110.0.
Fig. 11, 12. *Undulozonosporites blaoensis* n. fsp., *Pteridaceae,* prep. Blao-13; 19.9/104.1.
Fig. 13, 14. *Laevigatosporites haardti* (R. POT. et VEN. 1934) TH. et PF. 1953 subfsp. *haardtioides* W. KR. 1967. *Polypodiaceae,* prep. Blao-5; 18.1/114.9.
Fig. 15, 16. *Laevigatosporites nitidus* (MAMCZAR 1960) W. KR. 1967 subfsp. *blaoensis* n. subfsp., *Polypodiaceae,* prep. Blao-1; 21.3/117.2.
Fig. 17, 18. *Microfoveolatosporis microsculptus* n. fsp., prep. Blao-17; 15.5/111.7.
Fig. 19, 20. *Verrucatosporites vancampoae* n. fsp., *Polypodiaceae,* prep. Blao-15; 16.6/107.1.
Fig. 21, 22. *Verrucatosporites alienus* (R. POT. 1931) TH. et PF. 1953, *Polypodiaceae,* prep. Blao-10; 8.2/116.1.
Fig. 23, 24. *Verrucatosporites guinetii,* n. fsp., prep. Blao-5; 4.9/102.7.


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
20µ

2. *Laevigatosporites nitidus* (MAMCZAR 1950) W. KR. 1967 subfsp. *blaoensis* n. subfsp. (Planche I, fig. 15, 16).

Diagnose

L'exospore de 0,8 µm épaisse, la marque de tétrade est courte, 1/2 de la longueur de la spore.

Plus grande dimension: 45–53 µm.

Subfsp. type: Planche I, fig. 15, 16, prep. Blao-1; 21,3/117,2.

Derivatio nominis: de Blao, localité type.

Diagnose différentielle: l'exospore plus mince, et en premier lieu la marque de tétrade courte distinguent bien cette forme du L. nitidus (MAMCZAR 1960) W. KR. 1967 subfsp. nitidus.

Appartenance botanique probable: *Polypodiaceae.*

Fgen.: *Microfoveolatosporis* W. KR. 1959.

1. *Microfoveolatosporis microsculptus* n. fsp. (Planche I, fig. 17, 18).

Diagnose

Spores monolètes. La surface est finement microfovéolée. Le diamètre des fovéoles est de 0,5 µm environ. L'épaisseur de l'exospore est environ 1 µm. La marque de tétrade n'atteint pas le grand diamètre de la spore; les extrémités sont souvent divisées. Autour des laesurae il y a un bourrelet arrondi, assez mince, de 0,5 à 1 µm.

Plus grande dimension: 40—50 µm.

Holotype: Planche I, fig. 17, 18, prep. Blao-17; 15,5/111,7.

Derivatio nominis: de la sculpture fine.

Diagnose différentielle: la taille relativement petite, et l'exospore plus mince distinguent bien cette espèce de *M. neogranuloides* W. KR. 1967.

Appartenance botanique probable: *Pteropsida, Filicinae.*

Fgen.: *Verrucatosporites* TH. et PF. 1953.

1. *Verrucatosporites vancampoae* n. fsp. (Planche I, fig. 19, 20).

Diagnose

Spores monolètes. Surface finement verruqueuse, les éléments de l'ornementation sont plats, et de 1,5 à 2 µm de diamètre. L'épaisseur de l'exospore n'atteint jamais 1 µm. La marque de tétrade est relativement courte, r = 1/2 environ.

Plus grande dimension: 28—35 µm.

Holotype: Planche I, fig. 19, 20, prep. Blao-15; 16,6/107,1.

Derivatio nominis: en hommage à MME. M. VAN CAMPO, directeur du Laboratoire de Palynologie, Montpellier.

Diagnose différentielle: il y a une certain ressemblance avec *V. arctotertiarius* W. KR. 1967 subfsp. arctotertiarius, mais l'ornementation de notre espèce est plus fine que celle mentionnée précédemment. Les éléments de la sculpture du *V. microverrucatus* W. KR. 1967 sont plus marquées et l'exospore est plus épaisse que chez les spores du Blao.

Appartenance botanique probable: *Polypodiaceae.*

2. *Verrucatosporites alienus* (R. POT. 1931) TH. et PF. 1953, *Polypodiaceae* (Planche I, fig. 21, 22).

Il semble que ce soit un type cosmopolite des *Polypodiaceae.*

3. *Verrucatosporites guinetii* n. fsp. (Planche I, fig. 23, 24).

Diagnose

Spores monolètes. Sculpture verruqueuse, les éléments de la sculpture sont de 2 µm de haut et de 2 à 3 µm de large. L'épaisseur de l'exospore est de 2,5 µm environ.

La marque de tétrade est relativement longue, mais n'atteint jamais les extrémités de la spore, r = 4/5.

Plus grande dimension: 43—50 µm.

Holotype: Planche I, fig. 23, 24, prep. Blao-5; 4,9/102,7.

Derivatio nominis: en hommage à MONS. PH. GUINET.

Diagnose différentielle: les éléments de la sculpture et la taille plus petite de notre espèce nouvelle permettent de la distinguer aisément du *V. alienus* (R. POT. 1931) TH. et PF. 1953. Le *V. semiclavatus* AKYOL 1964 est beaucoup plus grand.

Appartenance botanique probable: *Polypodiaceae.*

Fgen.: *Dacrydiumites* COOKSON 1953.

1. *Dacrydiumites florinii* COOKSON et PIKE 1953, *Dacrydium* (Planche II, fig. 1—4).

Note. — Selon Potonié (1958) ce genre de forme est synonyme du *Phyllocladidites* (COOKSON 1947) COUPER 1953. Il est à noter que l'espèce florinii n'a pas été classé dans le genre *Phyllocladidites*. Il nous semble que pour résoudre ce problème il faut attendre des documents plus amples. Pour l'affinité botanique, voir le livre de WANG et al., (1960).

Fgen: *Pityosporites* SEWARD 1914.

1. *Pityosporites insignis* (NAUM. ex BOLCH. 1953) W. KR. 1971, *Abietaceae, Pinus* (Planche II, fig. 5, 6).

Note. — Suivant Huang (1972) ressemble à *Pinus massoniana.*

Fgen.: *Podocarpidites* (COOKSON 1947) R. POT. 1958.

Ici il y a lieu de citer le travail de SIVAK (1975): p. 358 "En effet la diversité des caractères morphologiques des calottes dans ces deux genres est telle qu'il faudrait pratiquement créer un type par espèce surtout pour le genre *Podocarpus.*" Il nous semble que les alvéoles qui ne forment pas une image de reticulation en coupe optique peuvent également fourni un des caractères des pollens des *Podocarpaceae.*

1. *Podocarpidites vancampoae* n. fsp. (Planche II, fig. 7, 8).

Diagnose

Sillon mince et allongé. L'exine est très mince, généralement moins de 1 µm d'épaisseur. Le tectum est perforé, le diamètre des perforations est d'environ 0,5 µm. La structure de la couche infratectale n'est pas observable au microscope optique. Le contour des ballonnets est demi circle. Le tectum est imperforé à ces endroits et de 0,5 µm d'épaisseur. La structure des alvéoles en coupe optique est irrégulière, "intrarugulée" seulement au bord du corps du grain, et disposé radialement. La longueur des éléments est de 1,5 à 2 µm environ.

Plus grande dimension: 55—65 µm.

Holotype: Planche II, fig. 7, 8, prep. Blao-5; 13,7/107,5.

Derivatio nominis: en hommage à MME. M. VAN CAMPO qui a beaucoup contribué à la connaissance des pollens des Gymnospermes.

Diagnose différentielle: la forme des ballonnets, le rapport du diamètre du grain et des ballonnets, séparent bien des espèces de forme européennes décrites dans le travail de KRUTZSCH (1971). Il y a une certain ressemblance avec le pollen du *Podocarpus gracilior* qui a été trouvé dans le Tertiaire supérieur d'Angola (KEDVES et SIMONCSICS (1978).

Appartenance botanique probable: *Podocarpaceae, Podocarpus nanhoensis* type (HUANG, 1972).

2. *Podocarpidites sivakii* n. fsp. (Planche II, fig. 9, 10).

Diagnose

Les ballonnets sont un peu plus grands que le corps du grain de pollen. Le sillon est long. La surface du grain est finement granuleuse. L'exine mesure de 1,5 à 2 µm d'épaisseur. L'épaisseur du tectum des ballonnets est d'environ 0,5 µm. Le système alvéolaire est irrégulier. Près du corps du pollen il y a une disposition radiale des éléments de la structure, mais les dimensions des alvéoles ne diffèrent pas sous le tectum, et à l'intérieur du ballonnet.

Plus grande dimension: 45—55 µm.

Holotype: Planche II, fig. 9, 10, prep. Blao-12; 18,4/115,0.

Derivatio nominis: en hommage à MONS. J. SIVAK pour sa contribution à la connaissance des pollens des Gymnospermes.

Diagnose différentielle: la taille plus petite et les ballonnets plus grands que le corps du grain distinguent bien cette espèce du *P. vancampoae* n. fsp.

Appartenance botanique probable: *Podocarpaceae*.

Fgen.: *Cupressacites* BOLCHOVITINA 1956.

1. *Cupressacites cuspidataeformis* (ZAKL. 1957) W. KR. 1971, ? *Taxaceae, Cupressaceae* (Planche II, fig. 11—14).

2. *Cupressacites insulipapillatus* (TREVISAN 1967) W. KR. 1971, *Juniperus* type (Planche II, fig. 15, 16).

Note. — La ressemblance des pollens du Viet-Nam avec les espèces de forme décrites d'Europe ne signifie sûrement pas l'identité des taxons botaniques.

Fgen.: *Inaperturopollenites* (PF. 1952 ex TH. et PF. 1953) emend. R. POT. 1958

1. *Inaperturopollenites vietnamiensis* n. fsp. (Planche II, fig. 17, 18).

Diagnose

Pollens sphériques à surface lisse. L'épaisseur de l'exine est d'environ 0,5 µm; au microscope optique on ne peut pas distinguer de couches internes.

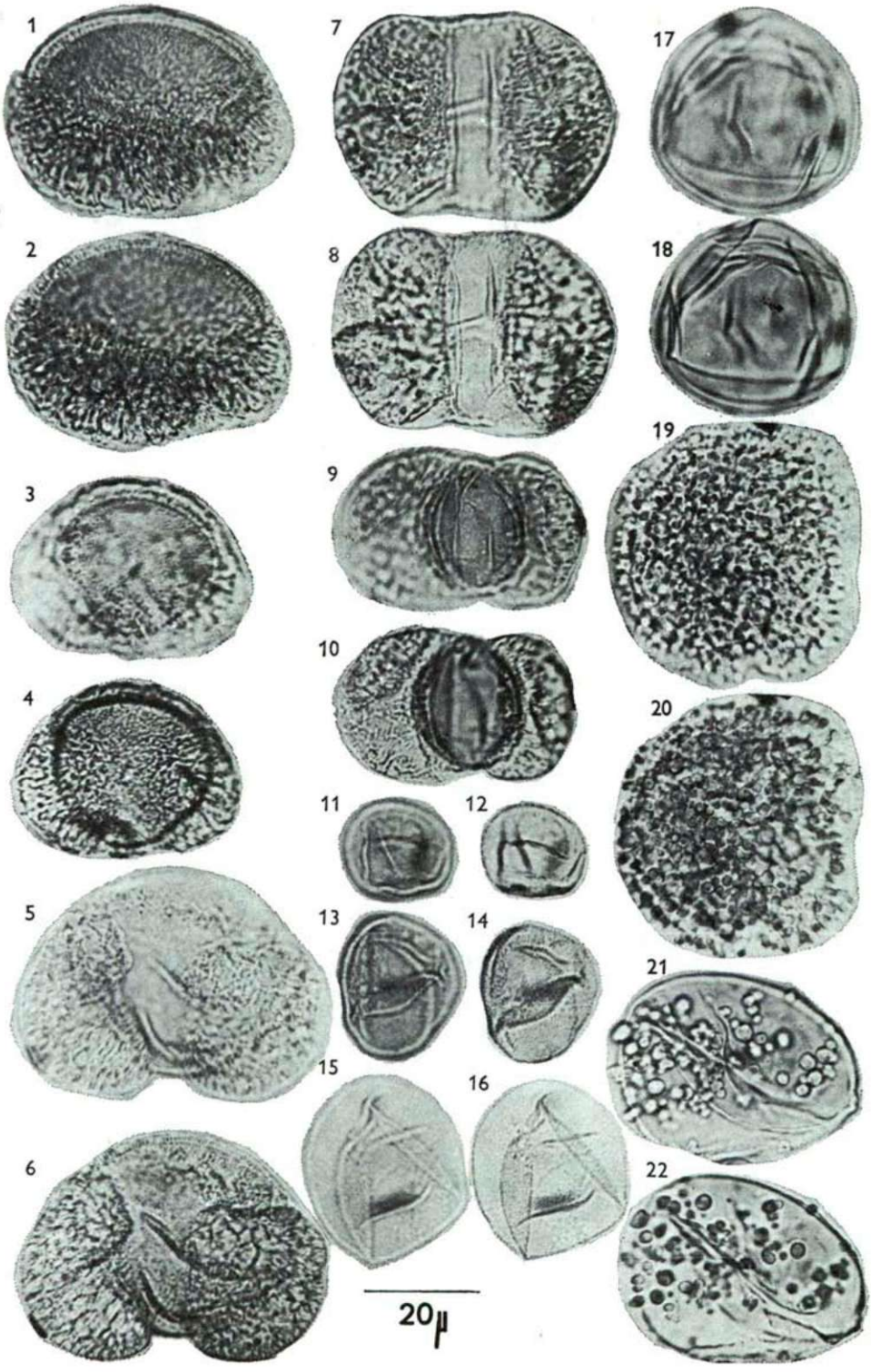
Plus grande dimension: 35—50 µm.

Holotype: Planche II, fig. 17, 18, prep. Blao-18; 18,3/114,6.

Derivatio nominis: du Viet-Nam.

Légende de la Planche II

Fig. 1, 2. *Dacrydiumites florinii* COOKSON et PIKE 1953, *Dacrydium*, prep. Blao-12; 6.3/109.5.
Fig. 3, 4. *Dacrydiumites florinii* COOKSON et PIKE 1953, *Dacrydium*, prep. Blao-5; 10.6/119.9.
Fig. 5, 6. *Pityosporites insignis* (NAUM. ex BOLCH. 1953) W. KR. 1971, *Abietaceae*, prep. Blao-3; 15.6/110.8.
Fig. 7, 8. *Podocarpidites vancampoae* n. fsp., *Podocarpaceae, Podocarpus nanhoensis* type, prep. Blao-5; 13.7/107.5.
Fig. 9, 10. *Podocarpidites sivakii* n. fsp., *Podocarpaceae*, prep. Blao-12; 18.4/115.0.
Fig. 11, 12. *Cupressacites cuspidataeformis* (ZAKL. 1957) W. KR. 1971, ? *Taxaceae, Cupressaceae*, prep. Blao-3; 11,2/118.4.
Fig. 13, 14. *Cupressacites cuspidataeformis* (ZAKL. 1957) W. KR. 1971, ? *Taxaceae, Cupressaceae*, prep. Blao-11; 9.3/115.3.
Fig. 15, 16. *Cupressacites insulipapillatus* (TREVISAN 1967) W. KR. 1971. *Juniperus* type, prep. Blao-17; 18.1/102.8.
Fig. 17, 18. *Inaperturopollenites vietnamiensis* n. fsp., prep. Blao-18; 18.3/114.6.
Fig. 19, 20. *Guinetia terciaria* n. fgen. et fsp., *Euphorbiaceae, Jatropha*, prep. Blao-15; 16.6/109,3.
Fig. 21, 22. *Guinetia terciaria* n. fgen. et fsp., *Euphorbiaceae, Jatropha*, prep. Blao-16; 21.8/108.7.


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
20μ

Diagnose différentielle : dimension plus petite par rapport au autres pollen types *Laricoides* fossiles.

Appartenance botanique probable: *Pseudotsuga* ou *Larix*.

Fgen.: *Guinetia* n. fgen.

Fgen. type: *Guinetia terciaria* n. fsp. (Planche II, fig. 19—22).

Note. — Il est à mentionner, que VAN DER HAMMEN (1954) a publié un type de pollen inaperturé avec sculpture gemmulée sous le nom *Pollenites typisimus*.

Diagnose

Formes inaperturées. La surface du paroi est couverte des éléments de l'ornementation de forme sphérique de différents diamètres. La sculpture est essentiellement gemmée.

Fgen. type: Planche II, fig. 19, 20, prep. Blao-15; 16,6/109,3.

Locus typicus: Blao.

Stratum typicum: couches houillières tertiaires.

Derivatio nominis: en hommage à MONS. PH. GUINET.

Diagnose différentielle: des formes inaperturées à surface gemmulée sont décrites ici pour le premier fois.

1. *Guinetia terciaria* n. fsp. (Planche II, fig. 19—22).

Diagnose

Contour en général circulaire, mais secondairement irrégulier ou elliptique. Le diamètre des gemmae est de 2 à 4 µm environ. L'épaisseur de l'exine (sans les éléments d'ornementation) est de 0,5 µm.

Plus grande dimension: 45—60 µm.

Holotype, locus typicus, stratum typicum, voir chez le genre de forme.

Derivatio nominis: de l'âge tertiaire des sédiments de la localité type.

Appartenance botanique probable: *Euphorbiaceae, Jatropha* (WANG et al., 1960; GUINET, 1962; LYNCH et WEBSTER, 1975).

Fgen.: *Graminidites* COOKSON 1947.

1. *Graminidites blaoensis* n. fsp. (Planche III, fig. 1, 2).

Diagnose

Contour circulaire, ou secondairement elliptique. Surface scabre à ponctuée. L'épaisseur de l'exine est toujours inférieur à 1 µm, l'ectexine se compose de trois couches qui sont de même épaisseur. Il y a un operculum; le diamètre du pore est de 1 à 1,5 µm, l'anulus est de 2 µm de large, mais très mince.

Plus grande dimension: 20—25 µm.

Holotype: Planche III, fig. 1, 2, prep. Blao-18; 20,1/113,8.

Derivatio nominis: du Blao, localité type.

Diagnose différentielle: c'est l'anulus, mince, qui sépare nettement cette espèce de *G. micropunctatus* W. KR. 1970 et de *G. gracilis* W. KR. 1970.

Appartenance botanique probable: *Gramineae*.

2. *Graminidites vancampoae* n. fsp. (Planche III, fig. 3, 4).

Diagnose

Contour circulaire, ou à cause de plis secondaires, de forme irrégulière. L'exine est très mince, de 0,3 à 0,5 µm. Surface ponctuée ou finement granuleuse. Le diamètre des pores est de 3 à 4 µm, il y a un operculum. L'anulus est de 2 µm de large environ.

Plus grande dimension: 40—50 µm.

Holotype: Planche III, fig. 3, 4, prep. Blao-13; 8,8/105,8.

Derivatio nominis: en hommage à Mme. M. Van Campo.

Diagnose différentielle: l'exine plus mince sépare nettement cette espèce de *G. pseudogramineus* W. Kr. 1970.

Fgen.: *Subtriporopollenites* Pf. et Th. 1953.

1. Cf. *Subtriporopollenites* fsp. (Planche III, fig. 5, 6).

Fgen.: *Triatriopollenites* Pf. 1953a.

1. *Triatriopollenites blaoensis* n. fsp. (Planche III, fig. 7, 8).

Diagnose

Contour triangulaire, côtés convexes. Surface finement ponctuée. L'exine, de 0,9 à 1,2 µm d'épaisseur, comprend trois couches d'égale épaisseur. L'épaisseur de l'anulus est 2 µm environ. L'atrium est granuleux.

Holotype: Planche III, fig. 7, 8, prep. Blao-15; 12,2/102,5.

Derivatio nominis: de Blao de la localité type.

Diagnose différentielle: l'exine mince distingue bien cette espèce du *Momipites microcoryphaeus* (R. Pot. 1931) Nichols 1973.

2. *Triatriopollenites desertorum* Kds. 1980, cf. *Myricaceae* v. *Juglandaceae* (Planche III, fig. 9, 10).

Note. — Il est intéressant que cette espèce, décrite des couches néogènes d'Egypte, soit présente dans les couches houillières de Viet-Nam.

Fgen.: *Compositoipollenites* R. Pot. 1960.

1. *Compositoipollenites potoniei* n. fsp. (Planche III, fig. 11, 12).

Diagnose

Contour triangulaire convexe ou quelquefois circulaire. Surface échinulée, scabré entre les épines. Le tectum est mince, la couche infratectale est columellaire. L'épaisseur du sole est égale à la hauteur des columelles. L'épaisseur de l'exine est de 0,7 à 1 µm environ. Les épines sont longues de 2—3 µm et élargies à leurs bases. Les pores sont subéquatoriaux, de 3 µm de diamètre. L'anulus est de 1—1,5 µm de large.

Plus grande dimension: 28—34 µm.

Holotype: Planche III, fig. 11, 12, prep. Blao-16; 6,4/113,6.

Derivatio nominis: à la mémoire de Mons. le Professeur R. Potonié, qui a fait connaître ces pollens.

Diagnose différentielle: la base des éléments sculpturaux forment une réticulation négative chez *C.* (al. *Pollenites*) *rizophorus* (R. Pot. 1934) R. Pot. 1960, ce qui distingue bien cette espèce de notre espèce de forme nouvelle.

Appartenance botanique probable: *Icacinaceae.*

2. *Compositoipollenites vancampoae* n. fsp. (Planche III, fig. 13, 14).

Diagnose

Contour triangulaire, convexe ou circulaire. L'épaisseur de l'exine est de 1 à 1,3 µm. Exine tectée perforée, à perforations très petites. Echinules de 2 à 3 µm de long s'élargissant à leurs bases. Structure baculée. Le diamètre des pores est de 3 µm environ. L'anulus mesure 3 µm de large et 3 µm d'épaisseur. Les pores sont en général subéquatoriaux.

Plus grande dimension: 40—48 µm.

Holotype: Planche III, fig. 13, 14, prep. Blao-15; 11,7/104,6.

Derivatio nominis: en hommage à Mme. M. Van Campo.

Diagnose différentielle: la taille plus grande distingue bien cette espèce de celles mentionnées précédemment.

Appartenance botanique probable: *Icacinaceae*.

3. *Compositoipollenites magnus* n. fsp. (Planche III, fig. 15,16).

Diagnose

Contour circulaire, secondairement plié et en forme d'ellipse. L'épaisseur de l'exine est de 1 à 1,3 µm environ. Tectum perforé, structure columellaire. La longueur des échinules est de 2 µm. Le diamètre des pores est de 3,5 µm environ, l'anulus mesure 4 µm de large et 3 µm d'épaisseur.

Plus grande dimension: 55—68 µm.

Holotype: Planche III, fig. 15, 16, prep. Blao-14; 13,8/112,3.

Derivatio nominis: de la taille grande à l'intérieur de ce genre de forme.

Diagnose différentielle: c'est la grande taille et les échinules rélativement petites, qui distinguent bien cette espèce des autres espèces de ce genre de forme.

Fgen.: *Malvacearumpollis* E. NAGY 1962.

1. *Malvacearumpollis nagyae* n. fsp. (Planche III, fig. 17, 18).

Diagnose

Contour circulaire. Le nombre des pores est de six en général. L'exine a 2 µm d'épaisseur elle est tectée perforée. Sur le tectum il y a des échinules est de grandes épines. La longueur des échinules n'atteint pas le 0,5 µm, les épines atteignent 3 µm. La couche infratectale est columellaire. La sole est la couche la plus épaisse parmi les couches de l'ectexine. Diamètre des pores de 4 à 5 µm, l'anulus est 3 µm large.

Plus grande dimension: 50—60 µm.

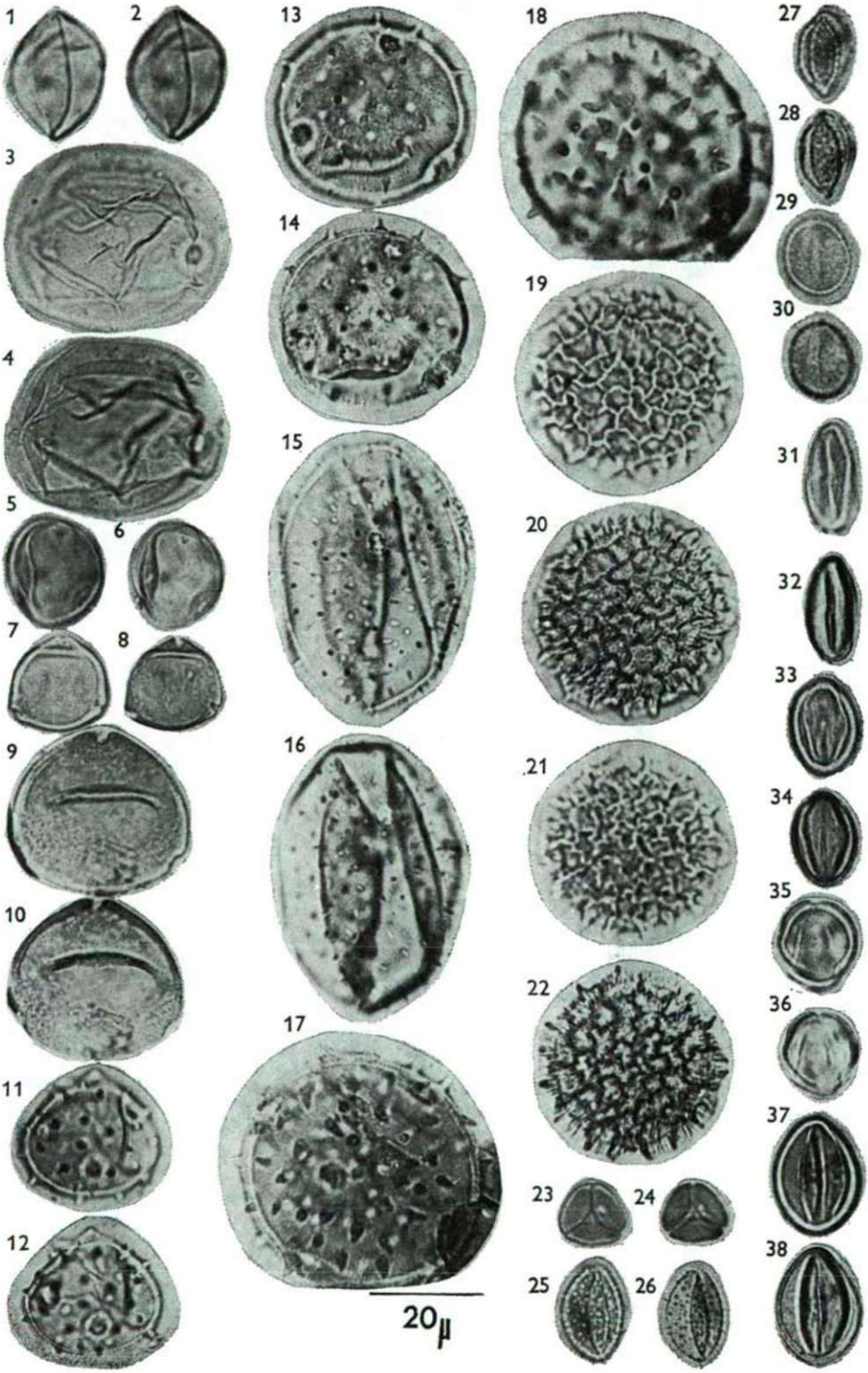
Holotype: Planche III, fig. 17, 18, prep. Blao-15; 9,3/102,9.

Derivatio nominis: en hommage à MME. E. NAGY, qui a décrit ce genre de forme.

Diagnose différentielle: le nombre des pores de l'espèce *M. estelae* (GER., HOPP. et MULL. 1968) n. comb., syn.: *Echiperiporites estelae* n. sp., in GER., HOPP.

Légende de la Planche III

Fig. 1, 2. *Graminidites blaoensis* n. fsp., *Gramineae*, prep. Blao-18; 20.1/113.8.
Fig. 3, 4. *Graminidites vancampoae* n. fsp., *Gramineae*, prep. Blao-13; 8.8/105.8.
Fig. 5, 6. Cf. *Subtriporopollenites* fsp., prep. Blao-18; 11.6/106.8.
Fig. 7, 8. *Triatriopollenites blaoensis* n. fsp., prep. Blao-15; 12,2/102.5.
Fig. 9, 10. *Triatriopollenites desertorum* KDS. 1980, cf. *Myricaceae*, prep. Blao-9; 15.0/113.2.
Fig. 11, 12. *Compositoipollenites potoniei* n. fsp., *Icacinaceae*, prep. Blao-16; 6.4/113.6.
Fig. 13, 14. *Compositoipollenites vancampoae* n. fsp., *Icacinaceae*, prep. Blao-15; 11.7/104.6.
Fig. 15, 16. *Compositoipollenites magnus* n. fsp., prep. Blao-14; 13.8/112.3.
Fig. 17, 18. *Malvacearumpollis nagyae* n. fsp., *Malvaceae*, prep. Blao-15; 9.3/102.9.
Fig. 19, 20. *Persicarioipollis meuseli* W. KR. 1962, *Polygonum*, prep. Blao-11; 11.3/116.3.
Fig. 21, 22. *Persicarioipollis meuseli* W. KR. 1962. *Polygonum*, prep. Blao-17; 10.3/107.8.
Fig. 23, 24. *Myrtaceidites parvus* COOKSON et PIKE 1954, *Myrtaceae, Eugenia* ou *Syzyngium* GAERTN., prep. Blao-12; 8.7/107.6.
Fig. 25, 26. *Feugueuripollenites blaoensis* n. fsp., *Palmae, Didymosperma*, prep. Blao-16; 21.9/105.7.
Fig. 27, 28. *Feugueuripollenites blaoensis* n. fsp., Palmae, *Didymosperma*, prep. Blao-1; 18.2/105.4.
Fig. 29, 30. *Feugueuripollenites blaoensis* n. fsp., Palmae, *Didymosperma*, prep. Blao-18; 17.4/107.5.
Fig. 31, 32. *Cupuliferoidaepollenites liblarensis* (THOMS. in POT., THOMS. et THIERG. 1950) R. Pot. 1960, *Fagaceae* v. *Leguminosae*, prep. Blao-2; 16.8/107.7.
Fig. 33, 34. *Scabratricolpites saurinii* n. fsp., *Oleaceae, Chionanthus* ou *Cornaceae, Helwigia*, prep. Blao-13; 20,0/107.9.
Fig. 35, 36. *Scabratricolpites saurinii* n. fsp., *Oleaceae, Chinanthus* ou *Cornaceae, Helwigia*, prep. Blao-17; 12,7/112.3.
Fig. 37, 38. *Scabratricolpites guinetii* n. fsp. *Fagaceae*, cf. *Quercus*, prep. Blao-5; 16.6/114.4.


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
20μ

et MULL. 1968, est de 20 à 24, ce qui la distingue nettement de notre espèce de forme nouvelle.

Appartenance botanique probable: *Malvaceae.*

Fgen.: *Persicarioipollis* W. KR. 1962.

1. *Persicarioipollis meuseli* W. KR. 1962, *Polygonum,* type *Persicaria* (Planche III, fig. 19—22).

Fgen.: *Myrtaceidites* COOKSON et PIKE 1954.

1. *Myrtaceidites parvus* COOKSON et PIKE 1954, *Myrtaceae, Eugenia* ou *Syzygium* GAERTN. (Planche III, fig. 23,24).

Fgen.: *Feugueuripollenites* KDS. 1968.

1. *Feugueuripollenites blaoensis* n. fsp. (Planche III, fig. 25—30).

Diagnose

Pollen fusiforme monosulqué, le sillon atteint les extrémités du grand axe du grain. L'épaisseur de l'exine est de 0,5 µm environ; les couches de l'ectexine ne sont pas observables au microscope optique. Surface échinulée lisse entre les épines; la longueur des épines est d'environ 1,5 µm.

Plus grande dimension: 15—25 µm.

Holotype: Planche III, fig. 25, 26, prep. Blao-16; 21,9/105,7.

Derivatio nominis: de la localité type.

Diagnose différentielle: le *F. eocaenicus* (KDS. 1965) KDS. 1968 est plus grand et à épines plus longues; le *F. microechinatus* KDS. 1968 est plus petit, et à épines plus courtes.

Appartenance botanique probable: Palmae, le genre *Didymosperma* est le plus probable, mais il est à noter que COUPER (1960) rapporté au genre *Astelia (Liliaceae)* un grain de pollen de l'Oligocène moyen qui ressemble un peu à notre espèce.

Fgen.: *Cupuliferoidaepollenites* R. POT. 1960 ex R. POT., THOMS. et THIERG. 1950.

1. *Cupuliferoidaepollenites liblarensis* (THOMS. in POT., THOMS. et THIERG. 1950) R. POT, 1960, *Fagaceae* v. *Leguminosae* (Planche III, fig. 31, 32).

Fgen.: *Scabratricolpites* (VAN DER HAMMEN 1956) GONZÁLEZ—GUZMÁN 1967.

1. *Scabratricolpites saurinii* n. fsp. (Planche III, fig. 33—36).

Diagnose

Contour ellipsoidal. Surface finement scabre. L'épaisseur de l'exine est de 1 à 1,5 µm, la couche infratectale est plus épaisse que le tectum et la sole. Structure intrabaculée. Les sillons en général atteignent les pôles et y fusionnent.

Plus grande dimension: 16—22 µm.

Holotype: Planche III, fig. 33, 34, prep. Blao-13; 20,0/107,9.

Derivatio nominis: en hommage à Mons. le Professeur SAURIN.

Diagnose différentielle: il y a une certain ressemblance avec S. tibialis GONZÁLEZ—GUZMÁN 1967, mais dans cette espèce l'ectexine est plus épaisse que l'endexine, et l'exine est épaisse dans la région équatoriale.

Appartenance botanique probable: *Oleaceae, Chionanthus* (WANG et al. 1960), ou *Cornaceae, Helwingia* (HUANG 1972).

2. *Scabratricolpites guinetii* n. fsp. (Planche III, fig. 37, 38).

Diagnose

Contour ellipsoidal, surface finement scabre ou ponctuée. L'épaisseur de l'exine est de 1,5 à 2 µm, les trois couches de l'ectexine sont grossièrement égales. Les sil-

lons n'atteignent pas toujours les pôles, en général l'un d'eux est plus court que les deux autres. Autour des sillons il y a des espaces vides de 1 μm de largeur environ.

Plus grande dimension: 20—28 µm.

Holotype: Planche III, fig. 37, 38, prep. Blao-5; 16,6/114,4.

Derivatio nominis: en hommage à Mons. Ph. GUINET.

Diagnose différentielle: la taille plus grande et l'exine plus épaisse distinguent nettement cette espèce de la précédente.

Appartenance botanique probable: *Fagaceae*, cf. *Quercus*.

à suivre

Adresse de l'auteur:
M. KEDVES
Institut botanique de l'Université Attila József
H-6701 Szeged, Boite Postal
657
Hongrie

Acta Biol. Szeged. 30, pp. 107–125 (1984)

THE EFFECT OF 2,4-D-CONTAINING HERBICIDE (DIKONIRT) ON THE ULTRASTRUCTURE OF CARP *(CYPRINUS CARPIO)* LIVER CELLS

I. BENEDECZKY[1], P. BÍRÓ[2] AND ZS. SCHAFF[3]

[1]*Attila József University, Department of Zoology, Szeged*
[2]*Limnological Research Institute at Lake Balaton, Hungarian Academy of Sciences, Tihany*
[3]*Ist Institute of Pathology and Experimental Cancer Research, Semmelweis Medical University, Budapest*

(Received June 30, 1983)

Abstract

The effect of sublethal concentrations (5, and 10 mg/l,) of 2,4-D-containing Dikonirt on the liver cells of carp, was studied by electronmicroscopy during an exposure of 6 months. Two months after treatment light swelling of the mitochondria, and fragmentation and loss of crystae were observed in the cytoplasm of the hepatocytes. A large number of inclusions containing electron dense bile pigment were detectable in the cytoplasm of the hepatocytes, and fine granular bile pigment appeared in the lumen of the bile canaliculi. The bile pigment being present simultaneously in the ductuli of the bile duct epithelial cells indicated that cholestasis was developing. The rough-surfaced endoplasmic reticulum tubuli displayed ring-formed fusion and paraprotein crystals were detected in certain dilated rEr cisternae. Later (after 3, 4, 5 months of exposure) inclusions similar to cholesterin crystals appeared in the cytoplasm, besides the cell alterations mentioned above; still later (in the 6th month) an increase in rough-surfaced endoplasmic reticulum and an appearance of fingerprints referred to an alteration of the protein-synthesizing system.

Key-words: Effect of 2.4-D on carp liver.

Introduction

The fish-ponds and natural waters of Hungary are loaded with communal and industrial sewages, chemical fertilizers, pesticides and detergents. The detection of the toxic agents and the diagnosis of the damaging effects caused by them on the aquatic organisms exposed to the increasing environmental pollution gain more and more emphasis. Many methods are at disposal for the demonstration of the damages developing in the aquatic organisms. Besides the classic toxicological studies, histopathological, biochemical and physiological methods are becoming all the more wide-spread. Thus, for example, the "normal" activity of various enzymes has been studied in the tissues, blood serum and plasma of certain fish species and their changes on environmental effects have been demonstrated (SIEBERT et al., 1964; MCBEAN et al., 1966; PEQUIN et al., 1970; GAUDET et al., 1975; MARQUEZ, 1976; SAURER and HAIDER, 1977, 1979). It has been unambiguously proved that even in the presence of low concentrations of toxic substances there is an increase in the activity of enzymes playing a role in the metabolism and the decomposition of toxic agents (BUHLER and BENVILLE, 1969; LANE and SCURA, 1970; RACICOT et al., 1975). Apart from the quantitative assays, not less significant are the histopathologic methods providing qualitative information, which supply important data to the localization and expansion of the tissue damages.

However, the light-microscopic histopathological alterations usually reflect the

"final result" of an irreversible vital process without giving adequate information in respect of the patho-mechanism. A further disadvantage of the light microscopic examination is the fact that it is only capable of detecting lethal (or perhaps sublethal) alterations caused by large doses; though, in the practice harmful effects of chemical substances being present in low concentration, but for long duration, (throughout months or years) are to be estimated.

In the viewpoint of attaining this object, besides the biochemical assays, the modern morphological methods, first of all electron microscopy, are becoming more and more significant. Electron microscopy is able to show, among others, incipient damages of cell organelles in due a time, when with the help of the biochemical assays no pathological alterations are demonstrable, e.g. in the blood. The acute effect of 2,4-D Na-salt on the early developmental stages of the bleak *(Alburnus alburnus)* has been reported elsewhere (BIRÓ, 1979).

In the present study, we wished to determine the effect of sublethal doses of the 2,4-D-containing herbicide Dikonirt on the ultrastructural organization of the hepatocytes of carps exposed for 6 months.

Materials and methods

Second-summer carps with 12.5–19.5 cm trunk lengths weighting 60–200 g, were used. The fish originating from fish-ponds were habituated to aquarium for a period of 4 months, and on one or two occasions weekly they received malachite green treatment until their susceptibility to Ichthyophthyrius infection ceased. Then the fish were divided into groups of 10 and kept in 100-1-sized glass aquariums in Dikonirt solutions of final concentrations 5 or 10 mg/l from February 14, 1980, till August 12, 1980 (2 parallel + 2 control groups). The water temperature was 15–17 °C. The fish were fed with living *Tubifex* and carp aliment. The water of the aquariums was strained with EHEIM filters filled with wad-strainer and the water was changed weekly. The water of the basins was ventilated with condensed air.

Five specimens were examined in each month. The animals were decapitated, their abdominal cavities opened, small slices were cut from various areas of the livers with a sharp safety razor, and the slices were immediately placed into ice-cold fixative. 2.5 % glutaraldehyde was used for prefixation, and that was buffered to pH 7.3 with cacodylate buffer. Following 2 h of prefixation the samples were fixed in 2 % OsO_4 solution for further 2 h. After dehydration in graded alcohol the samples were embedded in Spurr synthetic resin. The ultrathin sections were contrasted with lead and studied in the electronmicroscope.

Results

During the course of the 6 months' exposure no fish decay was observed either in the control group, or in those treated with Dikonirt.

Untreated control: On the basis of the overall view of low magnification (Fig. 1) spherical or kidney-shaped cell nuclei, poor in chromatin, were seen in the majority of the hepatocytes. The electron dense nucleolus was striking in the light karyoplasm. The cytoplasm of the liver parenchyma cells was moderately electron dense, many mitochondria, large amounts of glycogen granules, here and there lipid droplets and lysosomes, were observed in it. The rough-surfaced endoplasmic reticulum (rEr) was found mainly in lamellar form in large quantities in the hepatocytes (Fig. 1). In Fig. 2, of higher magnification, it can be seen that highly electron dense associated chromatin surrounded the nucleoli. The Golgi apparatus of the liver parenchyma cells was well-developed, with many vesicles, numerous granules and a few lysosomes in its area. Due to the double fixation, the glycogen granules occurring in the form

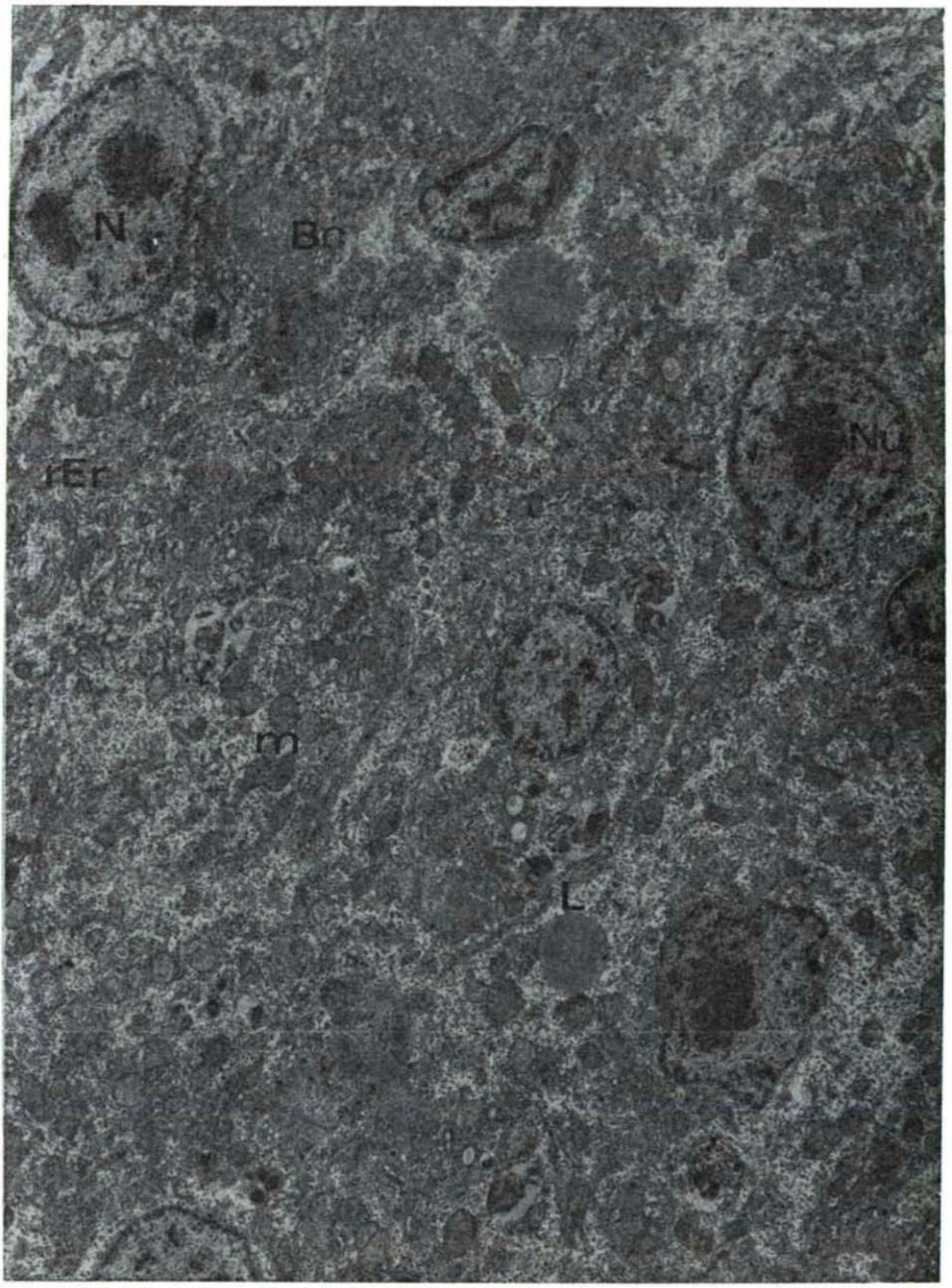


Fig. 1. Overall view of the liver parenchyma cells of an untreated carp. The nucleus (N) of the hepatocytes is poor in chromatin, the nucleolus (Nu) is striking. Many mitochondria (m), abundant rEr, moderate amount of glycogen granules can be found in the cytoplasms. L = lipid, Bc = bile canaliculus x 4400

of both monoparticles and rosettes appeared moderately electron dense (Fig. 2). The rEr tubuli were sometimes parallely arranged; they frequently formed shorter and small fragments and they were found all over the cytoplasm in an even distribution (Fig. 2). Tightly attached microvilli were found situated in the cavity of the bile canaliculi. A few cytoplasmatic inclusions were also detected in hepatocytes. A characteristic association of fine fibrous structures and electron dense amorphous tissue bundles was detected in these (Fig. 2).

Livers of fish exposed to 2,4-D (Dikonirt): The first sampling was performed two months following treatment. Macroscopically no striking alteration was seen in the livers. Light microscopically neither necrosis nor inflammation could be detected in the liver tissue. Ultrastructural alterations referring to the effect of the agent could not be detected in the cell nuclei in the electron micrographs, in the cytoplasm, however, ultrastructural signs referring to cell damage were observed in numerous cell organelles. Certain mitochondria, appeared to be light and swollen and crystae were fragmented or disappeared (Figs 3, 5, 6). In some areas mitochondria were encapsulated in autophage vacuoles (Fig. 3).

The cytoplasmic inclusions described in the normal liver, significantly increased in the hepatocytes (Fig. 4). The inclusions varied from 1 to 3 μm in diameter. Mostly filamentous substances accumulated in the inclusions, however, highly electron dense details were also regularly found in smaller numbers. The accumulation of electron dense matter was frequently observed in the wide lumen of bile canaliculi. In semithin sections this occurred in the form of a greenish-blue substance and corresponded to bile pigment, which indicated developing cholestasis (Fig. 7). Striking amounts of glycogen granules were found in certain hepatocytes, in which the amount of cell organelles decreased (Fig. 8). A frequent phenomenon was the ring-formed fusion of certain rEr tubuli as well as the presence of isolated cytoplasm detail and or a mitochondrion, in the lamella of the ring (Figs 3, 5, 6). Some rEr tubuli showed cisterna-like dilatations and a material of crystalline structure accumulated in the cisternae (Figs 6, 7). The accumulation of large amounts of bile pigment was often detectable in the ductuli of the bile duct epithelial cells (Fig. 9). The fine-structural appearance of this was rather similar to those observed in the cytoplasmic inclusions (Fig. 9).

The second sampling was performed on May 19, in the 3rd month of the treatment. The mitochondrial damages observed at the time of the first studying were still detectable, and as a new change of giant, elongated mitochondria appeared. Electron dense cytoplasmic inclusions were invariably observed in many hepatocytes (Fig. 10). Some of the inclusions resembled cholesterin crystals. Hepatocytes abundant, in cytoplasmic glycogen granules and poor in cell organelles were present in increased numbers (Fig. 8). The frequency of accumulation of bile pigment in the ductuli of the bile duct epithelial cells remained unchanged. The appearance of so-called "dark" and light cells was a new phenomenon in this period. At the time of the 3rd sampling (8 June) no new alterations were detectable, however, the amount of rEr considerably increased by the time of the last sampling (Fig. 11). Parallel with each other, long tubuli bundles were observable around the cell nucleus or at the peripheral part of the cytoplasm (Fig. 11). Fingerprints also appeared frequently (Fig. 12). The surface of the fingerprint membranes was generally found to be free of ribosomes (Fig. 12). The intermembranous cytoplasmic substance contained glycogen granules (Fig. 12). Occasionally, myelinization of fingerprint membranes could also be detected (Fig. 12).

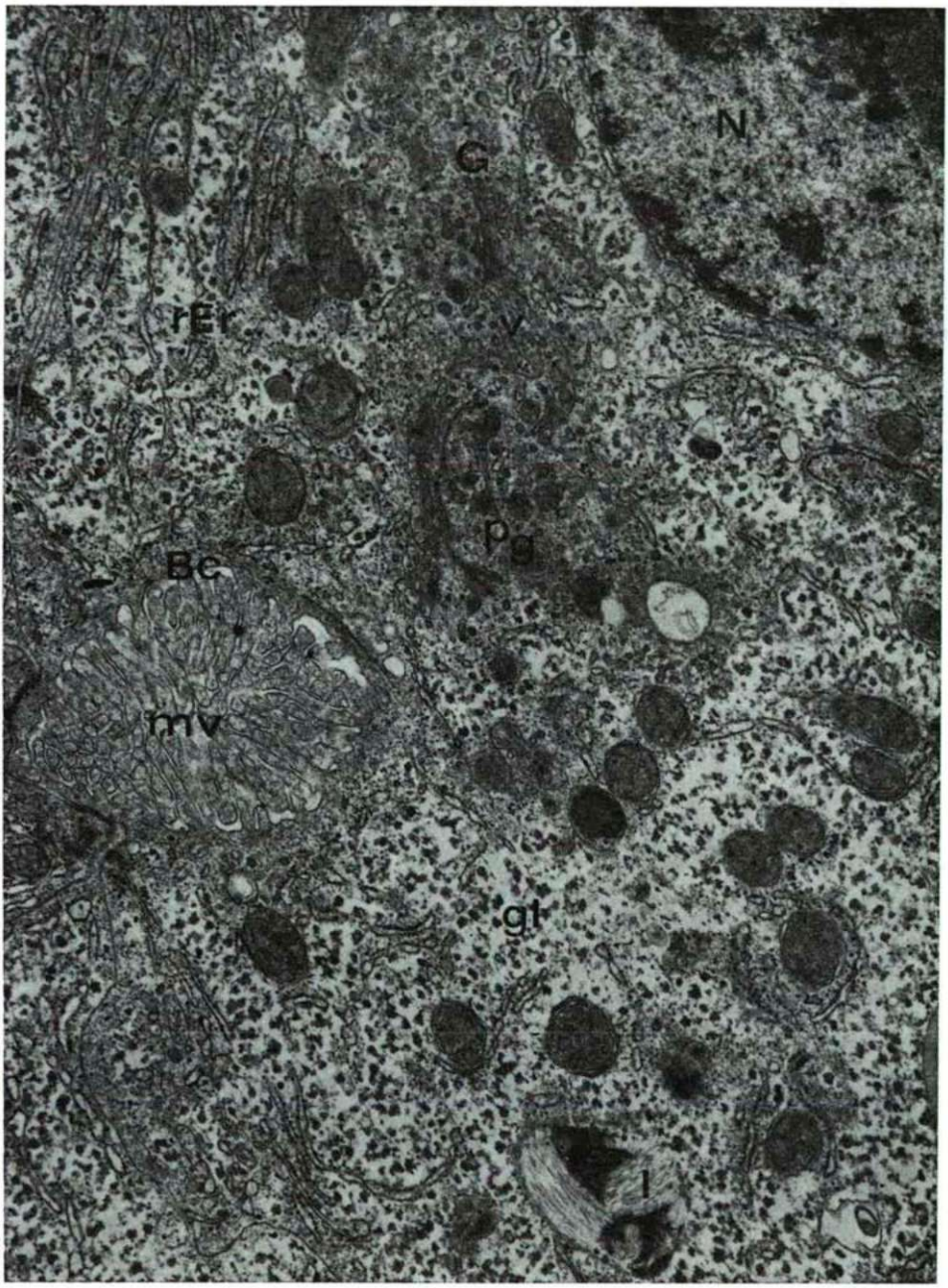


Fig. 2. Untreated control. Parenchymal cell. Well-developed Golgi apparatus (G) beside the cell nucleus (N). The Golgi area is rich in vesicular (v) elements and prosecretory granules (pg). The glycogen granules (gl) are evenly distributed in the cytoplasm. At places the tubuli of the rEr are slightly dilated. The lumen of the bile canaliculus (Bc) is abundant in microvilli (mv). I=inclusion x 12 000

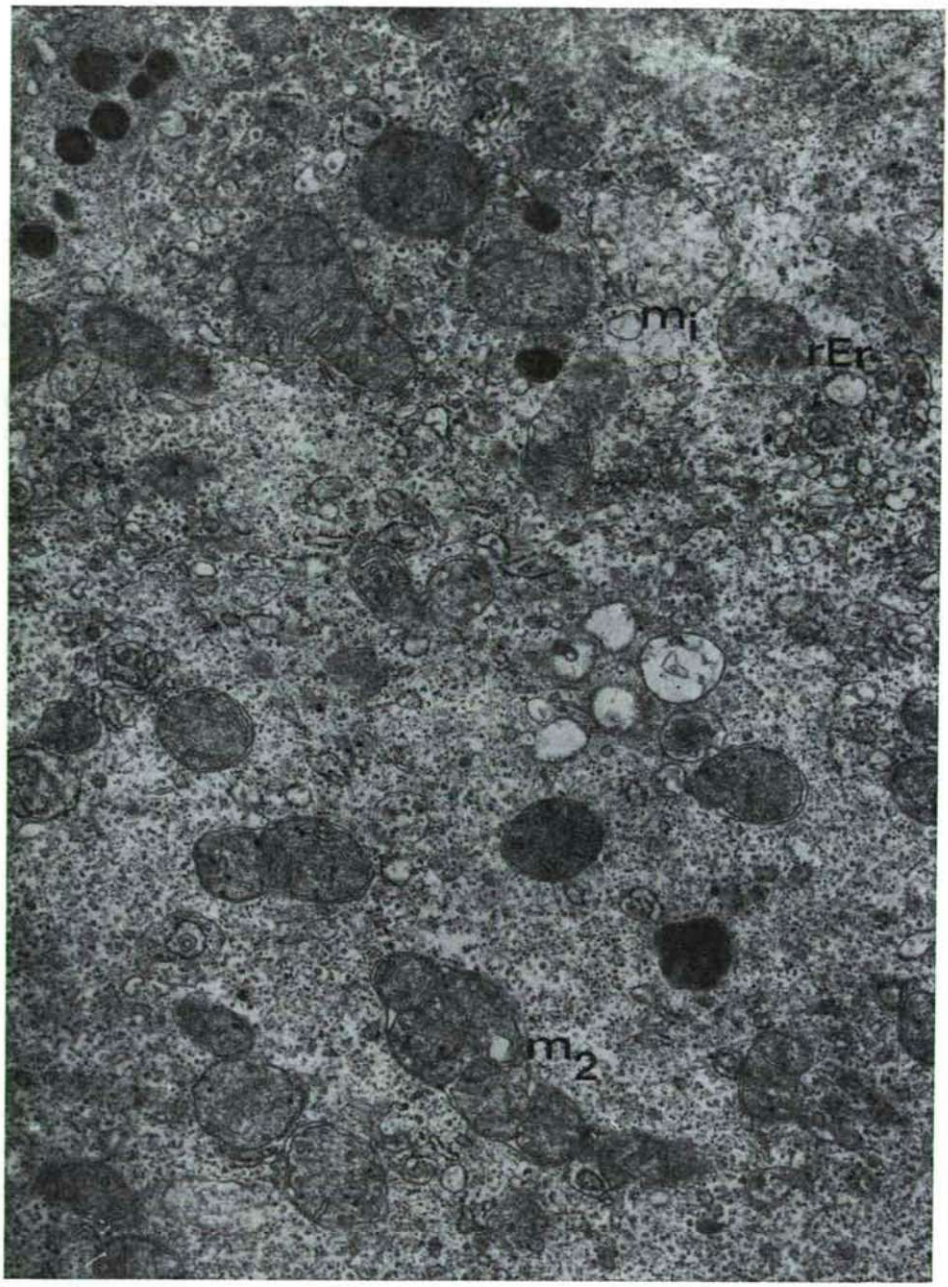


Fig. 3. Detail of a hepatocyte after 2 months of exposure to 2,4-D. Certain mitochondria (m_1) are strongly swollen, others (M_2) are closed in autophage vacuoles. The rEr is mainly present in vesicular form. x 13 000

Fig. 4. Hepatocyte after 2 months of exposure to 2,4-D. Many inclusions (I) in the cytoplasm. The inclusions are spherical, their inner substance is made up of fine fibrous material of varying the electron density. Note the strongly electron dense amorphous material in the inclusions beside the fibrous matter. x 8000

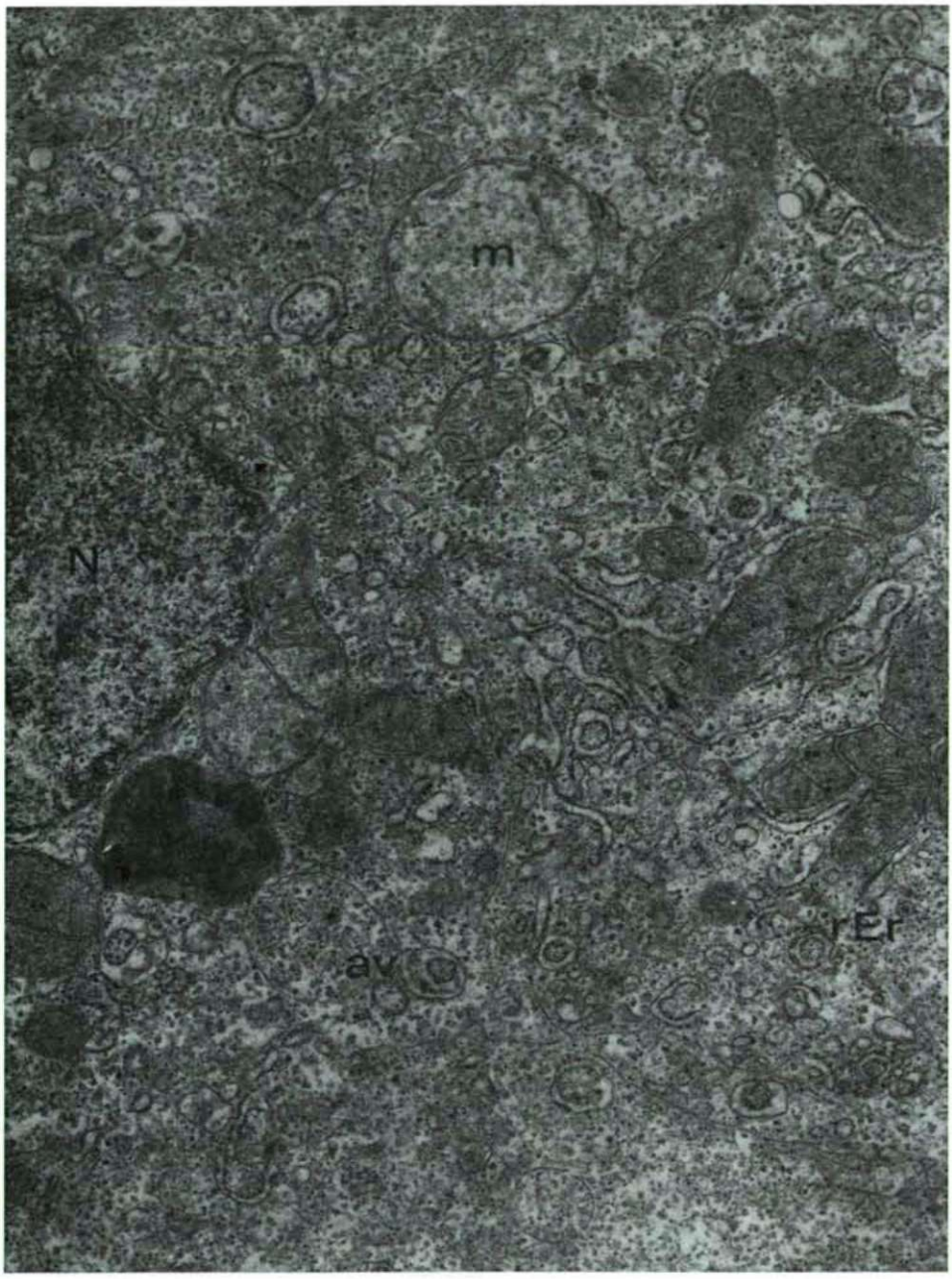


Fig. 5. Hepatocyte after 2 months of exposure to 2,4-D. Swollen mitochondria (m) and autophage vacuoles (av) limited by single or double membrane. The rEr has become transformed vesicularly. N=cell nucleus x 12 000

Fig. 6. Hepatocytes after 2 months of exposure to 2,4-D. Accumulation of crystalline substances in certain Er cisternae (→). m=swollen mitochondrium, N=cell nucleus x 20 000

Fig. 7. Hepatocytes after 2 months of exposure to 2,4-D. Accumulation of moderately electron dense material (bile pigment) in several bile canaliculi (Bc). The Golgi apparatus (G) has collapsed, no prosecretory granules can be found in its substance. rEr I = crystalline-like material in the Er cisternae x 10 000

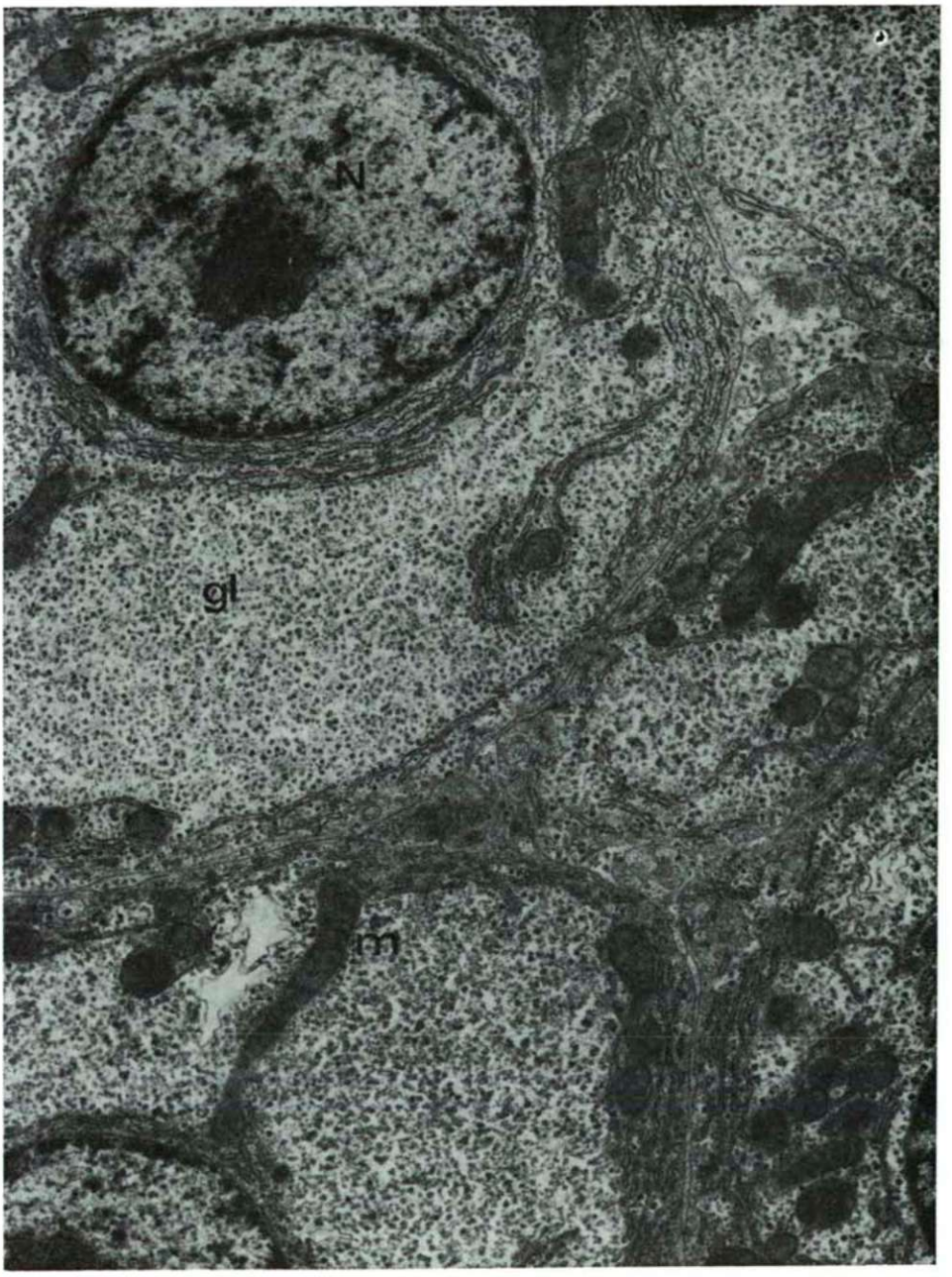


Fig. 8. Hepatocytes after 2 months of exposure to 2,4-D. Cell organelles have strikingly decreased in number in certain hepatocytes. The cytoplasm is mainly filled by glycogen (gl). N=cell nucleus, m=mitochondrion x 12 000

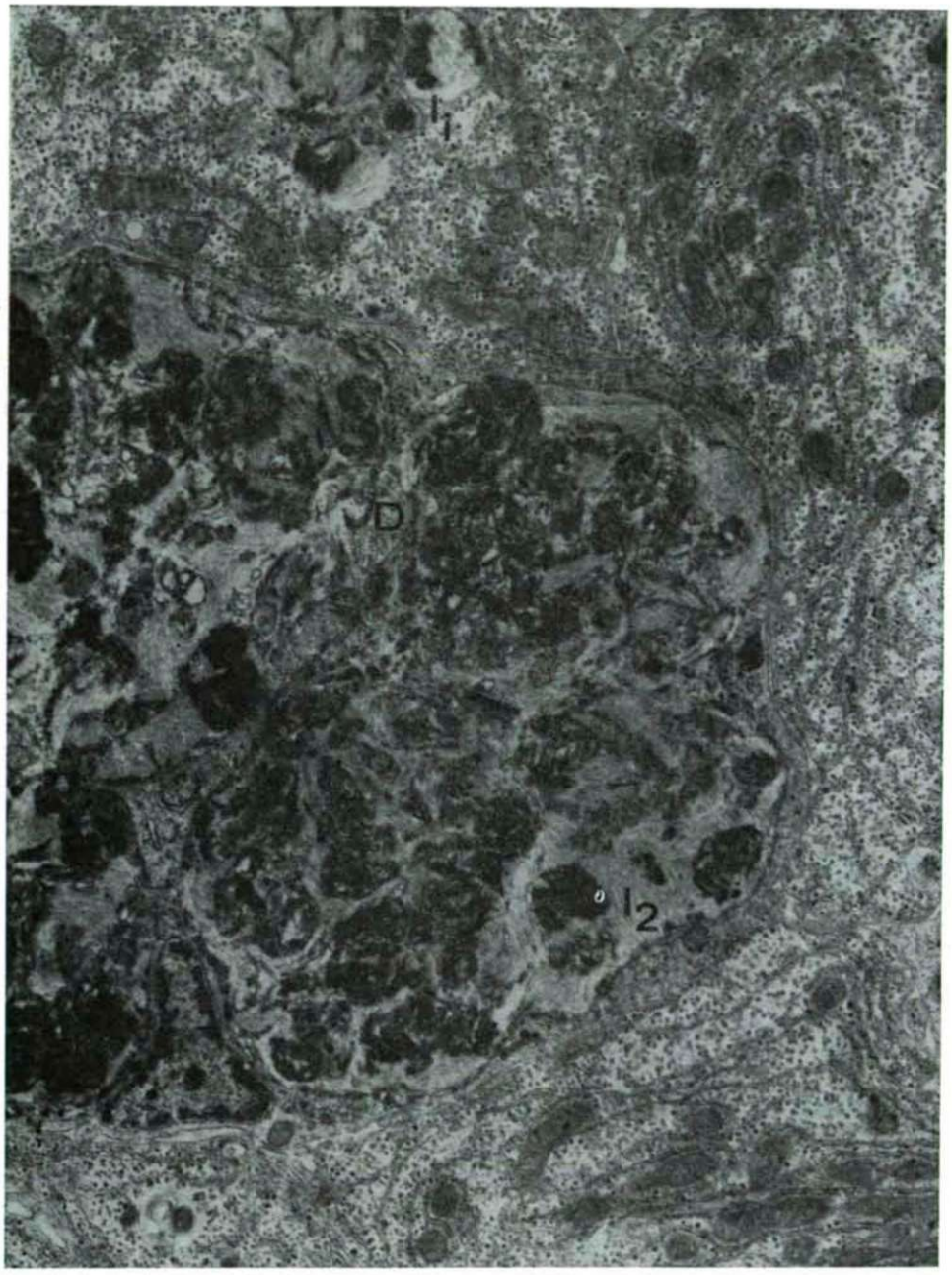


Fig. 9. Masses of inclusions (I_2), similar to those seen in the hepatocytes, frequently occurred in the bile duct (D). I = inclusion in the hepatocyte; 2 months after 2,4-D treatment x 8000

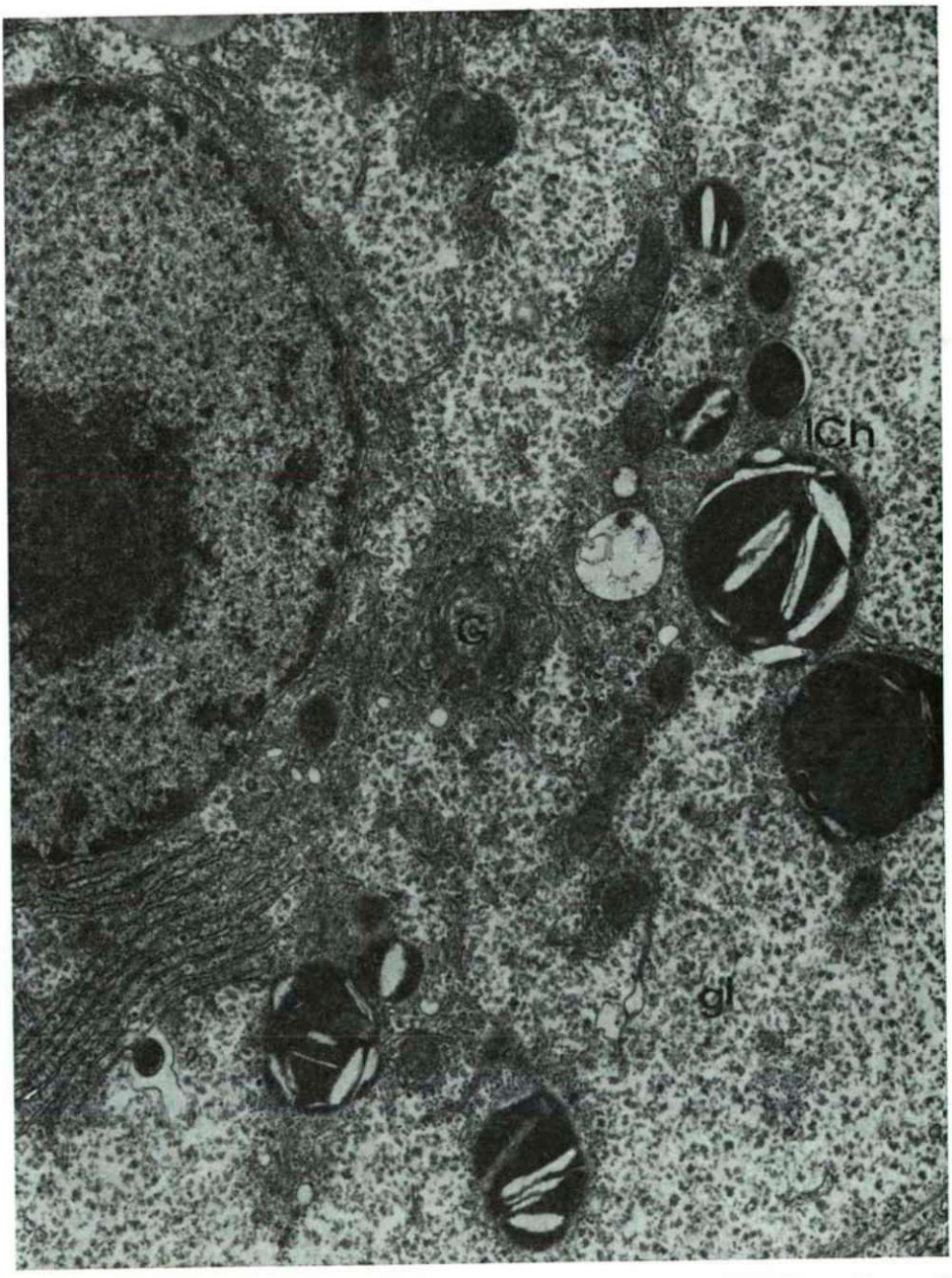


Fig. 10. Hepatocyte after 3 months of exposure to 2,4-D. Inclusions containing cholesterin crystals (ICh) in hepatocytes. The Golgi apparatus (G) has strikingly collapsed. The cytoplasm is rich in glycogen (gl). x 12 000

Fig. 11. Liver parenchyma cells after 6 months of exposure to 2,4-D. The cells are rather rich in rEr tubuli. 5–8 Er laminae (rEr) are frequently arranged as parallel bundles. x 4400

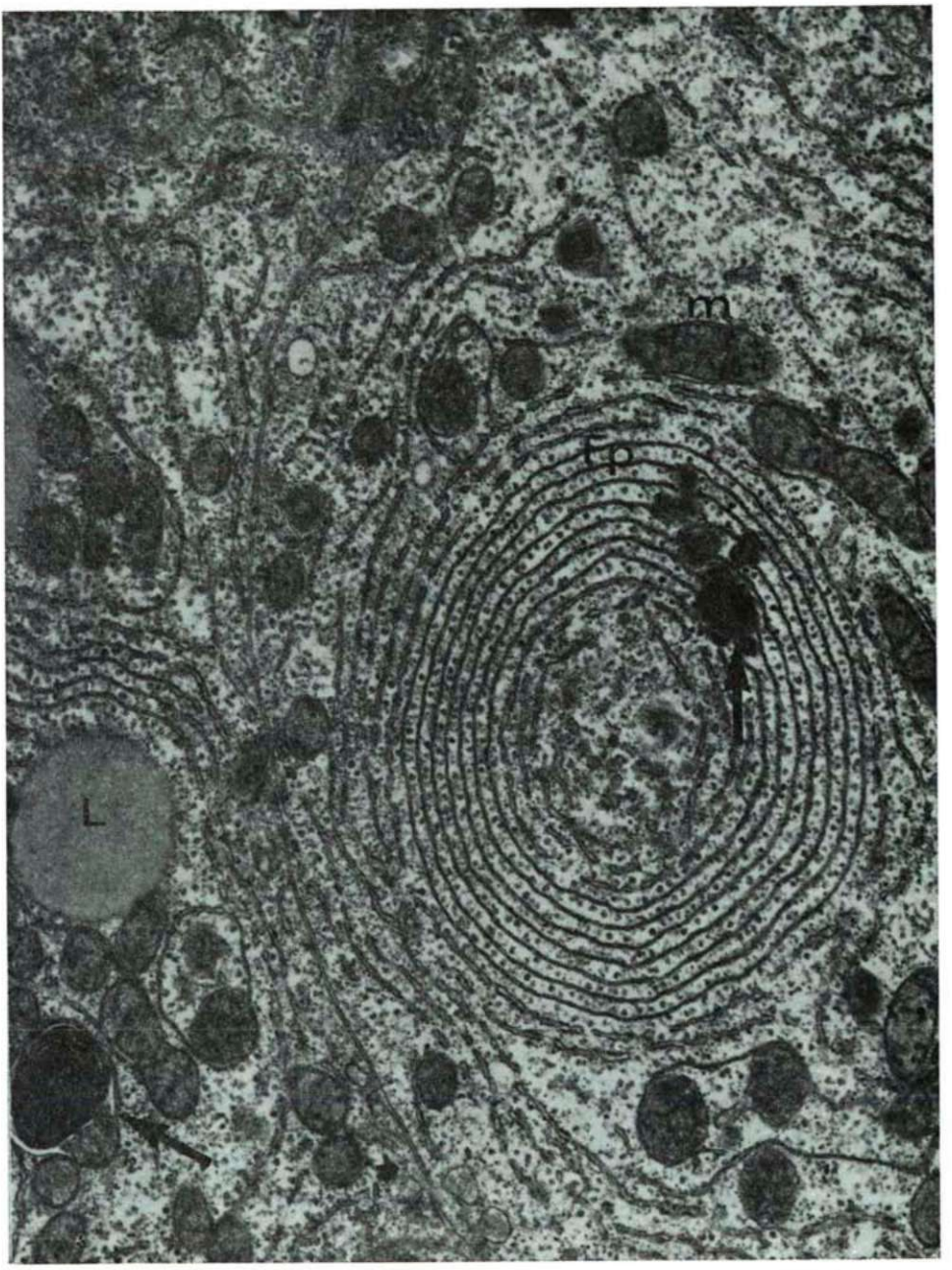


Fig. 12. Hepatocytes after 6 months of exposure to 2,4-D. The frequent appearance of "fingerprints" (Fp) was common in the hepatocytes. Note the myelinization of membranes in both the fingerprints (→) and certain mitochondria (m). L = lipid droplet x 12 000

Discussion

Salts and esters of 2,4-dichlorophenoxy acetic acid (2,4-D) have been utilized as herbicides in plant protection since long, and comprehensively (BORDÁS, 1967; BIRÓ, 1979; MCBRIDE et al., 1981).

In Hungary, a 2,4-D preparation named "Dikonirt" since the 'fifties it has been used regularly and in large amounts (BORDÁS, 1967) as selective herbicide.

In 1965, catastrophic fish decay was observed Lake Balaton (BARON et al., 1967). According to PÓNYI and PFEIFER (1979), it was caused by the chlorinated hydrocarbons demonstrated in the water. Although the cause of the fish decay in the Lake is still debated, there is no doubt that we must reckon with the occurrence of various contaminating chemical agents in natural waters and fish-ponds of Hungary. These agents may influence the metabolism of the flora and fauna of the waters even in low concentrations (TARZWELL, 1965; ASHLEY, 1972).

As to the occurrence of 2,4-D in Lake Balaton, PFEIFER et al. (1979) have shown that the agent is present from April to July generally in a concentration of 1—10 μg/l. At places even concentrations between 25 and 40 μg/l 2,4-D have been demonstrated in the Lake at places (PFEIFER et al. 1979). Since, due to occasional rain the concentration of 2,4-D may rise significantly in fish-ponds, the waters flowing into the Lake Balaton, and then in the Lake itself, it is important to estimate the effects of the sublethal doses of 2,4-D on the various aquatic organisms and the vital processes of the fish, in long-term experiments. It has been determined in regard to zooplankton that the amount of the most important filtering zooplankton of the Lake Balaton *(Eudiaptomus gracilis)* has considerably decreased compared to the 1930s (BANKÓS and PÓNYI, 1976; PÓNYI and PFEIFER 1979). PÓNYI and PFEIFER (1979) assume that the loading by 2,4-D-containing Dikonirt also has a role in the loss of the zooplankton. The acute toxic effect of the 2,4-D derivatives on the early developmental processes of fish has been reported by several authors (MOUNT and STEPHAN, 1967; KAMLER, 1972; BIRÓ, 1979). Sublethal doses of 2,4-D (5—50 mg/l) induced significant stress response in juvenile sockeye salmon (MCBRIDE et al., 1981). Histopathological alterations were observed by light microscopy in the branchiae, liver, and interrenal tissue of the kidney (MCBRIDE et al., 1981). Regarding the liver, MCBRIDE et al., (1981) determined that the tissue injury was focal, appearing in the form of vacuolar degeneration. Using second-summer carps, we failed to detect the alterations described by MCBRIDE et al., (1981) light microscopically; however, we were able to detect the presence of bile pigment proving the development of cholestasis, both in the cytoplasm of the liver cells and in bile ductuli. Cholestasis represents a severe disturbance of the bile secretory apparatus (DESMET, 1979), and this disturbance in liver function could be observed in the liver of the carps throughout the whole study period. Our electron microscopic studies support the diagnosis set up by light microscopy, since accumulation of electron dense bile pigment could be detected in both the hepatocytes and the lumen of the bile canaliculi. Accumulation of electron dense complex bile pigment was also observed by KENDREY (1980) in hepatocytes of a man working at a plant protection station. The development of paraprotein crystal-inclusions appearing in the rEr cisternae suggests that, apart from the damage of the bile-secretion, the transport of certain secretory proteins were also disturbed in the liver parenchyma cells of the fish exposed to 2,4-D. The development and persistence of the large amounts of cytoplasmic inclusions found in the hepatocytes can

practically be explained by an inhibited transport. The fine fibrous substance in the secondary lysosomes was structurally similar to the material found in the residual bodies accumulated in the bile ducts. Presumably, the accumulation of bile pigment in the cytoplasm of the cells and bile ducts resulted from disturbed transport processes, similarly to the human diseases accompanied by cholestasis (DESMET, 1979; SCHAFF and LAPIS, 1979).

Besides the accumulation of paraprotein crystals, further alterations occurred in the rEr. The vesicularization of the rEr tubuli, as well as their degranulation at places, also speaks in favour of the assumption that the exposure to 2,4-D considerably affected the protein synthesizing system, too. Since the rEr tubuli often cease being longitudinally arranged under energy-deficient circumstances (SCHAFF and LAPIS, 1979), it may be assumed that 2,4-D exerts its effect on the protein-synthesizing rEr elements through the mitochondrial system (ZVIRGZDS et al., 1971; KAMLER et al., 1974). We emphasize that the rEr was damaged throughout the whole period of exposure, nevertheless, the damage was more striking in the early and middle periods: in the last period, on the other hand, a significant rEr increase took place. The degenerative alterations were followed by a regenerative process, the cell-physiological background of which is unknown. The fact that "fingerprints" also appeared besides the increase in Er supports the view that 2,4-D affects the ribosome-Er-membrane relationship, i. e., it makes ribosomes detach from the Er-membrane, thus, affecting the process of protein synthesis (BUTLER, 1979; DAVIDSON, 1979).

A significant elevation of glutamate dehydrogenase activity was measured by BIRÓ in the blood of 2,4-D treated fish (BIRÓ, 1981, unpublished data). This increase in enzyme activity is consistent with the ultrastructural alterations referring to the mitochondrial damages (light swelling, disappearance of crystae, autophagy) detected by us. The enzyme may easily escape from the swollen, damaged mitochondria, and may become diffused in the blood in large quantities through the vascular pole of the hepatocytes. On the basis of the presence of damaged mitochondria, the consequence was drawn that the energy-supply of the cells was not satisfactory. In such a manner, the structural damage of the mitochondria may serve as an explanation also for the interpretation of the inhibited transport processes (cholestasis) discussed above.

In conclusion, exposure to 2,4-D for a period of 6 months produced ultrastructural cell damages in the hepatocytes suggestive of *(i)* a disturbance in the energy-supply of the cells, *(ii)* an inhibition of transport processes *(iii)* an alteration of the entire cell metabolism. There is no doubt that electronmicroscopic cytopathology can be applied successfully in the environment and nature conservancy research and in the elimination and prevention of the developed damages.

References

ASHLEY, L. M. (1972): Nutritional Pathology. In Fish Nutrition (J. E. HALVER, ed.). — New York, London: Academic Press, 439–537.

BANKÓS, L. and PÓNYI, J. (1976): Studies on the effect of a few more important pesticides at Lake Balaton and on the aquatic invertebrates from Séd at Aszódfő. Conference at Lake Balaton. Lake Balaton, No. *3/14*, 1–16.

BARON, F., CSONTI, F. and PÓNYI, J. (1967): Investigations of pesticide residues in fish and other aquatic organisms of Lake Balaton and some other aquatic habitats. — Annal. Biol. Tihany *34*, 117–128.

BÍRÓ, P. (1979): Acute effects of the sodium salt of 2.4-D on the early developmental stages of bleak, *Alburnus alburnus*. — J. Fish. Biol. *14*, 101–109.

BORDÁS, S. (1967): Dangerous pesticides. — Budapest, Agricultural Publishing House, pp. 720.

BUHLER, D. R. and BENVILLE, P. (1969): Effect of feeding and of DDT on the activity of hepatic glucose 6-phosphate dehydrogenase in two salmonids. — J. Fish. Res. Bd. Can. *26*, 3209–3216.

BUTLER, W. H. (1979): Experimental liver injury. — in: Pathology of the liver. eds.: MACSWEEN, R. N. M., ANTHONY, P. P., SCHEUER, P. J., CHURCHILL LIVINGSTONE. Edinburgh, London, New York, 55–68.

DAVIDSON, C. S. (1979): Pathophysiology of liver. — in: Pathology of the liver. eds.: MACSWEEN, R. N. M., ANTHONY, P. P., SCHEUER, P. J., CHURCHILL LIVINGSTONE. Edinburgh, London, New York, 32–55.

DESMET, V. J. (1979): Cholestasis; extrahepatic obstruction and secundary biliary cirrhosis. — in: Pathology of the liver. eds.: MACSWEEN, R. N. M., ANTHONY, P. P., SCHEUER, P. J., CHURCHILL LIVINGSTONE. Edinburg, London, New York, 272–306.

GAUDET, M., RACICOT, J. G. and LERAY, C. (1975): Enzyme activities of plasma and selected tissues in rainbow trout *Salmo gairdneri* RICHARDSON. — J. Fish. Biol. *7*, 505–512.

KAMLER, E. (1972): Bioenergetical aspects of the influence of 2.4-D-Na on the early development stages in carp (*Cyprinus carpio* L.). — Pol. Arch. Hydrobiol. *19*, 451–474.

KAMLER, E., MATLAK, O. and SROKOSZ, K. (1974): Further observations on the effect of sodium salt of 2.4-D on early developmental stages of carp (*Cyprinus carpio* L.). — Pol. Arch. Hydrobiol. *21*, 481–502.

KENDREY, G. (1980): The role of the pathologist in studies on the alterations of the liver caused by pesticides. Communications of the Heavy Chemical Industry Research Institute, Veszprém, *10*, 235–247.

LANE, D. E. and SCURA, E. D. (1970): Effects of dieldrin on glutamic oxalacetic transaminase in *Poecilia latipinna*. — J. Fish. Res. Bd. Can. *27*, 1869–1871.

MARQUEZ, E. D. (1976): A comparison of glutamic-oxalacetate transaminase, lactate dehydrogenase, α-hydroxybutyrate and creatin phosphokinase activities in mon-spawning, pre-spawning and spawning pink salmon. — Comp, Biochem. Physiol. *54B*, 121–123.

MCBEAN, R. L., NEPPEL, M. J. and GOLDSTEIN, L. (1966): Glutamate dehydrogenase and ammonia production in the eel *(Anguilla rostrata)*. — Comp. Biochem. Physiol. *18*, 909–920.

MCBRIDE, J. R., DYE, H. M. and DONALDSON, E. M. (1981): Stress response of juvenile sockeye salmon *(Oncorhynchus nerka)* to the butoxyethanol ester of 2.4-dichlorophenoxyacetic acid. — Bull. Environm. Contam. Toxicol. *27*, 877–884.

MOUNT, D. I. and STEPHAN, C. E. (1967): A method for establishing acceptable toxicant limits for fish-malathion and butoxyethanol ester of 2.4-D. — Trans. Am. Fish. Soc. *96*, 185–193.

PEQUIN, L., PARENT, J. P. and VELLAS, F. (1970): La glutamate dehydrogenase chez la carpe (*Cyprinus carpio* L.) .Distribution et role dans l'ammoniogenese. — Archs. Sci. Physiol. *24*, 315–322.

PFEIFER, GY, PÓNYI, J. E. and NAGY, Z. (1979): Pesticide residues in Lake Balaton. — Symp. Biol. Hung. *19*, 21–26.

PÓNYI, J. and PFEIFER, GY. (1979): The effect of pesticides on the aquatic ecosystems. Communications of the Heavy Chemical Industry Research Institute, Veszprém, *9*, 209–231.

RACICOT, J. G., GAUDET, M. and LERAY, C. (1975): Blood and liver enzymes in rainbow trout (*Salmo gairdneri* RICH.) with emphasis on their diagnostic use: study of CCl_4 toxicity and a case of *Aeromonas* infection. — J. Fish. Biol. *7*, 825–835.

SAUER, D. M. and HAIDER, G. (1977): Enzyme activities in the serum of rainbow trout, *Salmo gairdneri* RICHARDSON; the effect of water temperature. — J. Fish. Biol. *11*, 605–612.

SAUER, D. M. and HAIDER, G. (1979): Enzyme activities in the plasma of rainbow trout, *Salmo gairdneri* RICHARDSON; the effects of nutritional status and salinity. — J. Fish. Biol. *14*, 407–412.

SCHAFF, Zs. and LAPIS, K. (1979): Cholestasis. — in: Electron Microscopy in Human Medicine *8*, The liver. The gallbladder and biliary ducts. ed:. JOHANNESSEN, J. V., MCGRAW-HILL, New York, 89–123.

SIEBERT, G., SCHMITT, A. and BOTTHE, I. (1964): Enzymes of the amino acid metabolism in cod musculature. — Arch. Fish. Wiss. *15*, 233–244.

TARZWELL, C. M. (1965): The toxicity of synthetic pesticides to aquatic organisms and suggestion for meeting the problem. — Ecology and the Industrial Society, Oxford: Blackwell, 197–218.

ZVIRGZDS, J. K., LACE, Z. M., GRUNDULE, M. V. and ZUJKA, A. A. (1971): (Effect of 2.4-D-Na on oxidative phosphorylation, mechano-chemical changes and adenosintriphosphatase activity of mitochondria of carp liver). — In Eksperimental'naja Vodnaja Toxikologija, Riga: Zinatne, *2*, 12–25.

Adress of the authors:
PROF. DR. I. BENEDECZKY
Department of Zoology
A. J. University
H-6701 Szeged P.O. Box. 659.
Hungary

Acta Biol. Szeged. 30, pp. 127–141 (1984)

COMPARATIVE ULTRASTRUCTURAL STUDY ON THE NEUROMUSCULAR JUNCTION IN THE MUSCLES OF THE ALIMENTARY CANAL OF *LOCUSTA MIGRATORIA* AND *HELIX POMATIA*

KATALIN HALASY and I. BENEDECZKY

Attila József University, Department of Zoology, Szeged

(Received June 30, 1983)

Abstract

The ultrastructure of the neuromuscular junctions in the gastrointestinal system of the migratory locust *(Locusta migratoria migratorioides)* and the snail *(Helix pomatia)* was studied comparatively.

Large amounts of synaptic and non-synaptic nerve terminals were found in the insect gut muscle. In the snail intestinal wall on the other hand, where the axolemma tightly fitted to the sarcolemma, the nerve terminals did not form neuromuscular synapses.

In both species, high numbers of non-synaptic terminals were found in which neurosecretory granules of varying electron density were present. Authors regard these terminals as peptidergic ones.

In the authors' opinion, the dense-cored axon terminals occurring in lower numbers are aminergic terminals.

Neurons were not detected in the insect, gut however, neurons containing peptidergic as well as those containing aminergic granules were found in the snail gut.

Key words: Neuromuscular junction in the gut of locust and snail

Introduction

Studies on the morphological characteristics, innervations and neuromuscular junctions of the muscular system of the gastrointestinal tract are mainly limited to the mammalian (rat, guinea-pig, rabbit, etc.), including human intestinal canal (GABELLA, 1979; GORDON—WEEKS, 1981, 1982; LLEWELLYN—SMITH et al., 1981; FAUSSONE—PELLEGRINI, 1983). Only sporadic data have been reported for other vertebrates, e. g. SALIMOVA and FEHÉR (1982), using microspectrofluorimetric and electron-microscopic methods, studied the innervation of the alimentary tract in chondrostean fish.

As to the neuromuscular junction of invertebrates, mostly Arthropods, among them a few insect species, viz. *Periplaneta americana, Locusta migratoria, Schistocerca gregaria,* etc., have been studied. ANDERSON et al., (1977, 1978) described neuromuscular synapses in the outer layer of the three-layered striated gut muscle of *Schistocerca gregaria.* BENEDECZKY and MILLER (1983) carried out studies on the aminergic and peptidergic innervation of the hindgut of *Periplaneta americana* and *Locusta migratoria.* ECKERT et al., (1981), applying an immunocytochemical method, demonstrated a peptidergic transmitter, the proctolin (BROWN, 1975), in the hindgut of the cockroach.

There are few literary data concerning other invertebrate phyla in respect to the neuromuscular junction (TÁNCZOS and TÁNCZOS, 1979; ÁBRAHÁM, 1983). The *Mol-*

lusca phylum, for example, is worthy of note, since the intestinal canal of the included species, similarly to the vertebrate intestinal canal, contains smooth muscle, the muscular system of the alimentary canal of Arthropods, on the other hand, is striated as insect muscles in general.

Since the gut muscle of different structure serves the same physiological functions in both phyla, it seemed justified to compare the gut muscle of a representative of each phylum (*Arthropoda* and *Mollusca*), with particular reference to the ultrastructure of the neuromuscular junctions.

Materials and methods

Mature male and female individuals of *Locusta migratioria migratorioides (Arthropoda: Insecta)* and *Helix pomatia (Mollusca: Gastropoda)* were used. The migratory locusts were obtained from the breed of the Biological Research Institute of the Hungarian Academy of Sciences, Tihany. The snails were collected in humid meadows, near Szeged. The animals were dissected and 1 mm^3 pieces cut from various parts of the alimentary tract were fixed in cold state in 2.5% glutaraldehyde (diluted with cacodylate buffer) for 2h at pH 7.3. Following this, the tissue blocks were washed in cacodylate buffer containing 7.5% sucrose. After washing, the material was postfixed in 2% osmium tetroxide, buffered with s-collidin (pH: 7.3) at +4 °C for 2h, then dehydrated in an ascending ethanol series. The tissue blocks were contrasted for 1h in 75% ethanol with saturated uranyl acetate. The blocks were embedded in Spurr embedding matter, and sections were prepared. The sections were contrasted with lead citrate, and photographs were taken in a TESLA BS 500 electron microscope.

Results

Both the striated gut muscle of *Locusta migratoria* and the gut smooth muscle of *Helix pomatia* are rich in nerves (Figs 1, 2). The sarcoplasm on the gut muscle fibres of *Locusta migratoria* (Fig. 1) forms processes of regular arrangement. The interstitial space surrounding the muscle fibres contains large amounts of collagen fibres; tracheoblasts and smaller tracheoles occur at places. Nerve fibres and terminals are also situated there. The nerve fibres are surrounded by glial processes, the axon terminals are often surrounded by the processes of the muscle fibres. The smooth muscle fibres are of varying courses in the gut muscle layer of *Helix pomatia* (Fig. 2). Omega-profiles and vesicles referring to exocytosis are rather frequent at the rims of the muscle fibres. The nerve bundles and nerve fibres run parallel with, or perpendicularly to the muscle fibres in the interstitial space, which is rich in collagen fibres. The membranes of the terminals are in tight connection with the sarcolemma at places, and here too, the nerve fibres not associated with muscle fibres are covered by processes of glial cells.

On the basis of the fine-structural characteristics of the granules and vesicles found in the nerve fibres and terminals, it can be stated that several kinds of fibre types and axon terminals play a role in the innervation of the muscles in both species.

Synaptic (Figs 3—5) and non-synaptic (Figs 6—7) nerve terminals can be distinguished in *Locusta migratoria*. The axon terminals forming synapsis always contain clear vesicles around 50 nm in diameter. The majority of the vesicle population of these terminals are formed by clear vesicles (Figs 3, 5); in other presynaptic elements, (Fig. 4), besides the clear vesicles, there are larger (diameters of 130 nm) granules of variable density (Fig. 4) in great numbers. Neuromuscular junction may

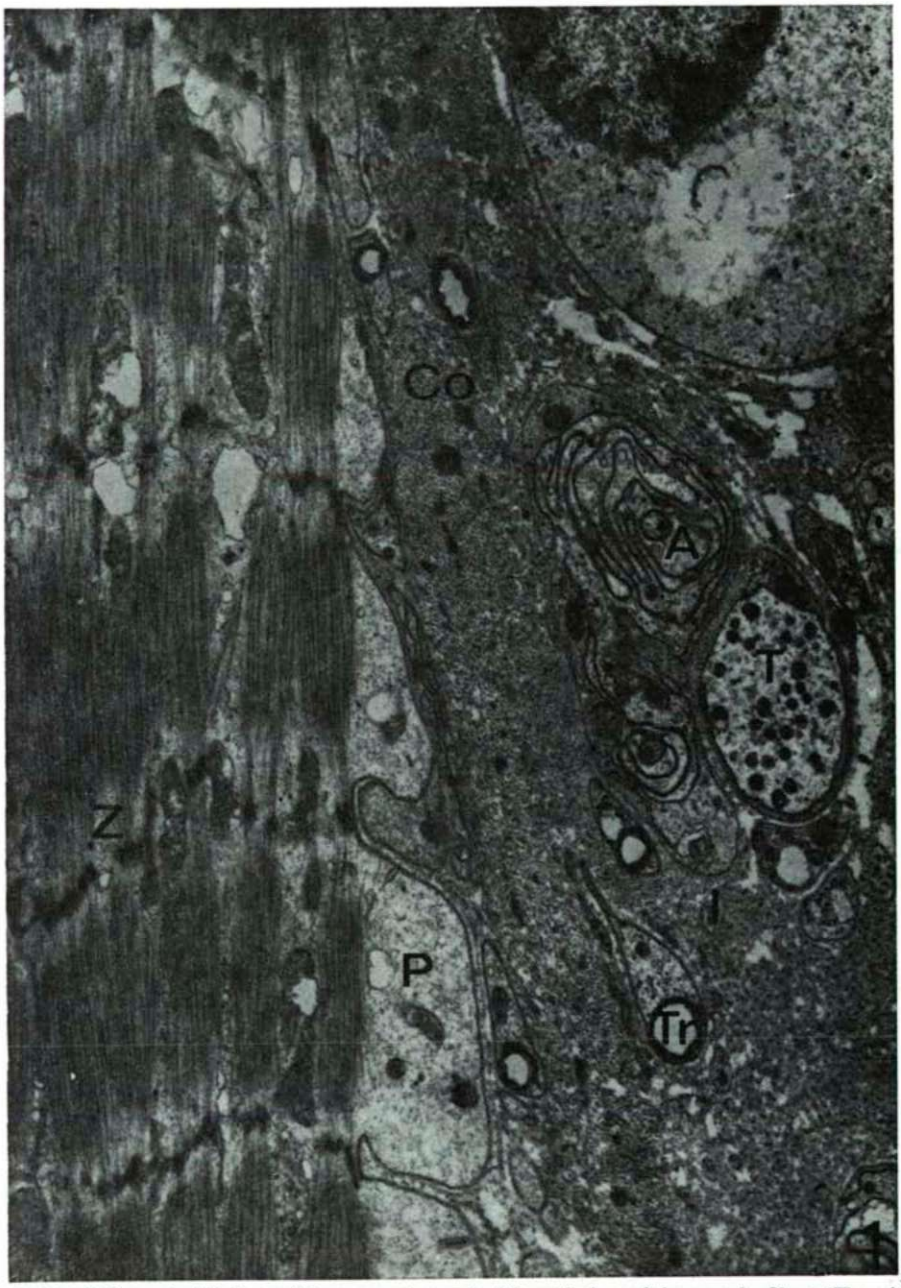


Fig. 1. Detail from the hindgut of *Locusta migratoria*. The striation of the muscle fibres (Z) and the protrusion (P) of the sarcoplasm can well be seen. Note the many collagen fibres (Co), various types of axons (A), and axon terminals (T) in the wide interstitium (I). Tr = tracheola

x 10,000

Fig. 2. Longitudinal section from the smooth muscle layer of snail gut. Besides the nerve bundles (NB) running parallel with the smooth muscle fibres (Mf), nerve fibres (N) running perpendicularly to the muscles are seen. Latter form tight morphological connection (arrow) with the muscle fibres at places. Co = collagen x 9900

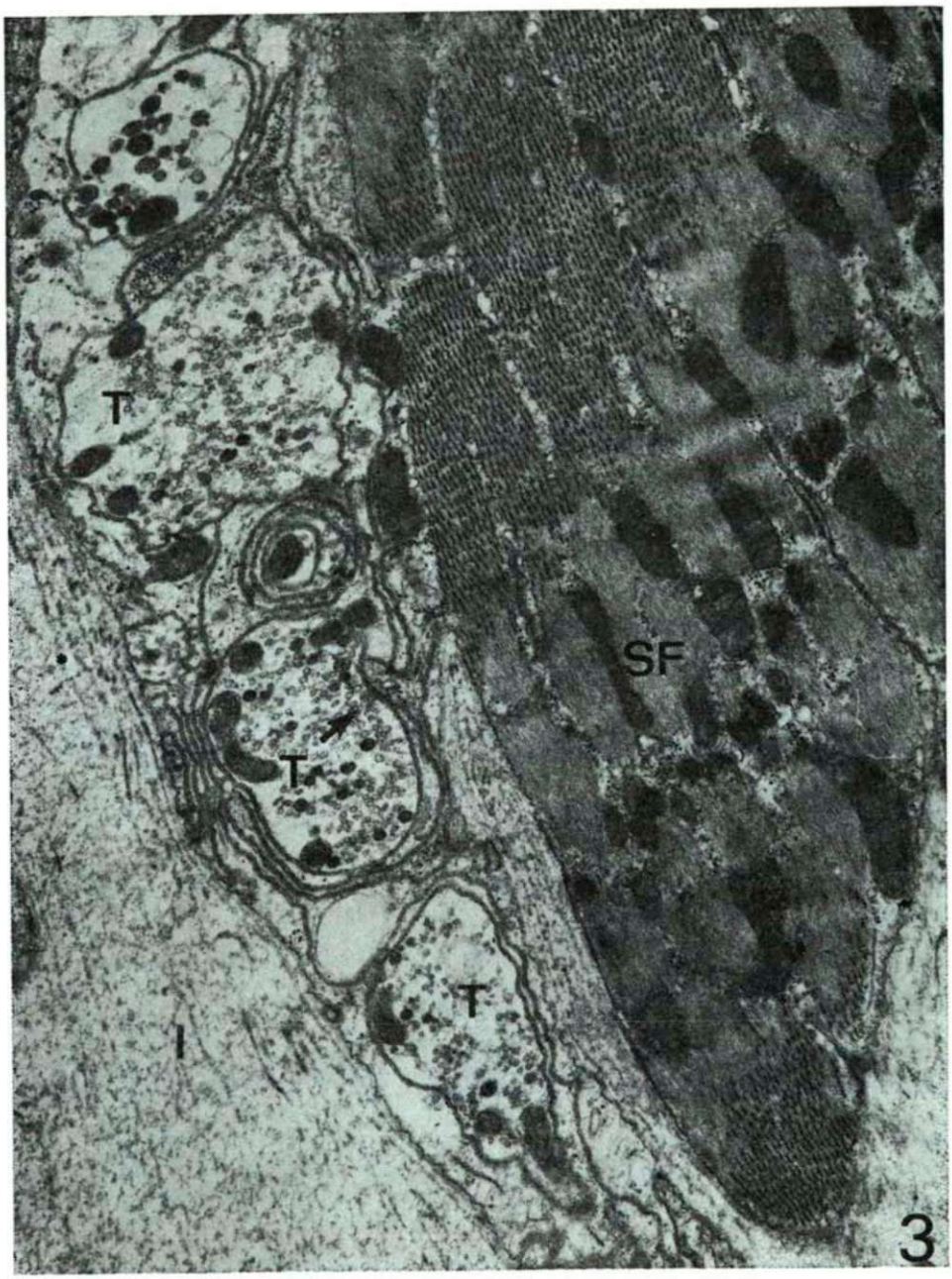


Fig. 3. The axon terminals (T) in the neighbourhood of striated muscle fibres (SF) of *Locusta migratoria* hindgut mostly contain clear vesicles and develop neuromuscular synapsis (arrow) with muscle processes. I = interstitium x 16,000

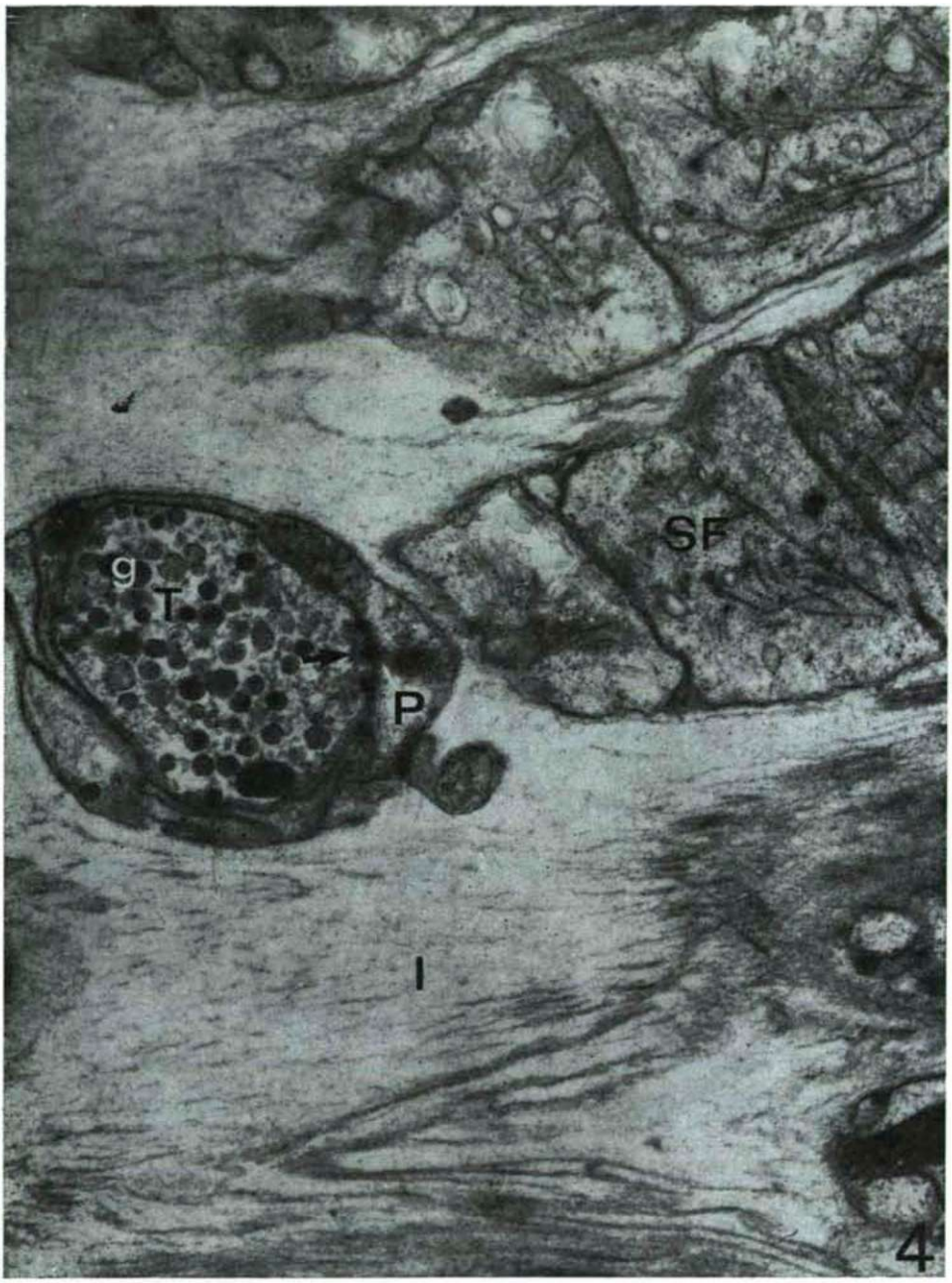


Fig. 4. Synapsis (arrow) between the process (P) of a striated muscle (SF) of *Locusta migratoria* hindgut and a terminal (T) which contains, apart from the clear vesicles, a great number of large, moderately electron dense granules (g). I=interstitium x 16,000

Fig. 5. High magnification of a neuromuscular junction in the gut musculature of *Locusta migratoria*. SF=striated muscle fibre; T=nerve terminal; C=accumulation of vesicles along the presynaptic membrane-thickening. x 70,000

develop between a process of the muscle and the axon terminal (Figs 3, 4), or the axon terminal adjacent to the sarcolemma of the muscle fibre forms a synapsis (Fig. 5). The other, non-synaptic, group of the nerve fibres contains only few clear vesicles, if at all, the terminals are filled by large (150 nm), highly electron dense granules (Figs 6, 7,). These also lie in the direct neighbourhood of the muscle fibres, often adjacent to the sarcolemma, however, synaptic relation cannot be observed in the case of this terminal type.

Several kinds of nerve fibres can be found among the gut muscle fibres of *Helix pomatia,* too. Fibres and terminals containing large (200 nm in diameter), highly electron dense granules occur here, too (Fig. 2, 8). The electron dense granules are mostly of ellipsoidal shape, in contrast to the spheroid form of the granules in insects. The electron density of the granules is also more variable than in the non-synaptic terminals of *Locusta migratoria:* moderately and highly electron dense granules are regularly found within the same terminal (Figs 2, 8). Two further types of nerve fibres occur in *Helix pomatia,* viz, those having so-called large (100 nm in diameter) dense-core vesicles (Fig. 8), and terminals containing moderately electron dense granules with large diameters (250 nm) and granular matrix (Figs 2, 9). The latter type occurs less frequently than the former. The latter two fibre types can be found, in the insect gut rarely. All three listed nerve fibre types may establish tight morphological contact with the muscle fibres (Figs 2, 8, 9), however, membrane specialization characteristic of the neuromuscular synapsis and accumulation of clear vesicles were not observed at all in the snail gut.

Not only nerve fibres, but even neurons were detected among the muscle fibres of *Helix pomatia* (Fig. 10). The perikaryon of the neurons is almost full of electron dense, variously shaped, mostly ovoid, granules. The cell nucleus is rich in chromatin, and besides the granules, the most characteristic organelles in the perikaryon are tubules of the rough-surfaced endoplasmic reticulum. Apart from the relatively small mitochondria, myeline figures and vacuoles occur in the cytoplasm of the neurons.

Discussion

During our comparative ultrastructural studies on the neuromuscular junctions of the *Locusta migratiora* and *Helix pomatia* guts, two basic differences were observed: First, in the snail gut we have not found neuromuscular junction referring to chemical impulse transmission in the classical sense.

Second, the uni-, bi- and multipolar neurons being present in significant amounts in the snail gut were absent in the insect gut.

It is difficult to give an unambiguous explanation for the two morphological differences. The fact that the gut wall of the snail is formed by smooth muscles is phylogenically a morphological and functional feature characteristic of vertebrates. The organization of the rich innervation observed by light microscopy in the snail gut muscle resembles the AUERBACH's plexus of vertebrates in many respects. In the AUERBACH's plexus, as well as in the snail gut muscle, the axon terminals do not form synaptic connection with the sarcolemma. In the insect gut muscle, on the other, hand, the many synaptic connections are noteworthy. These are not completely identical in shape with the skeletal muscle's neuromuscular motor end plates of vertebrates, but the pre- and postsynaptic thickening can well be identified here

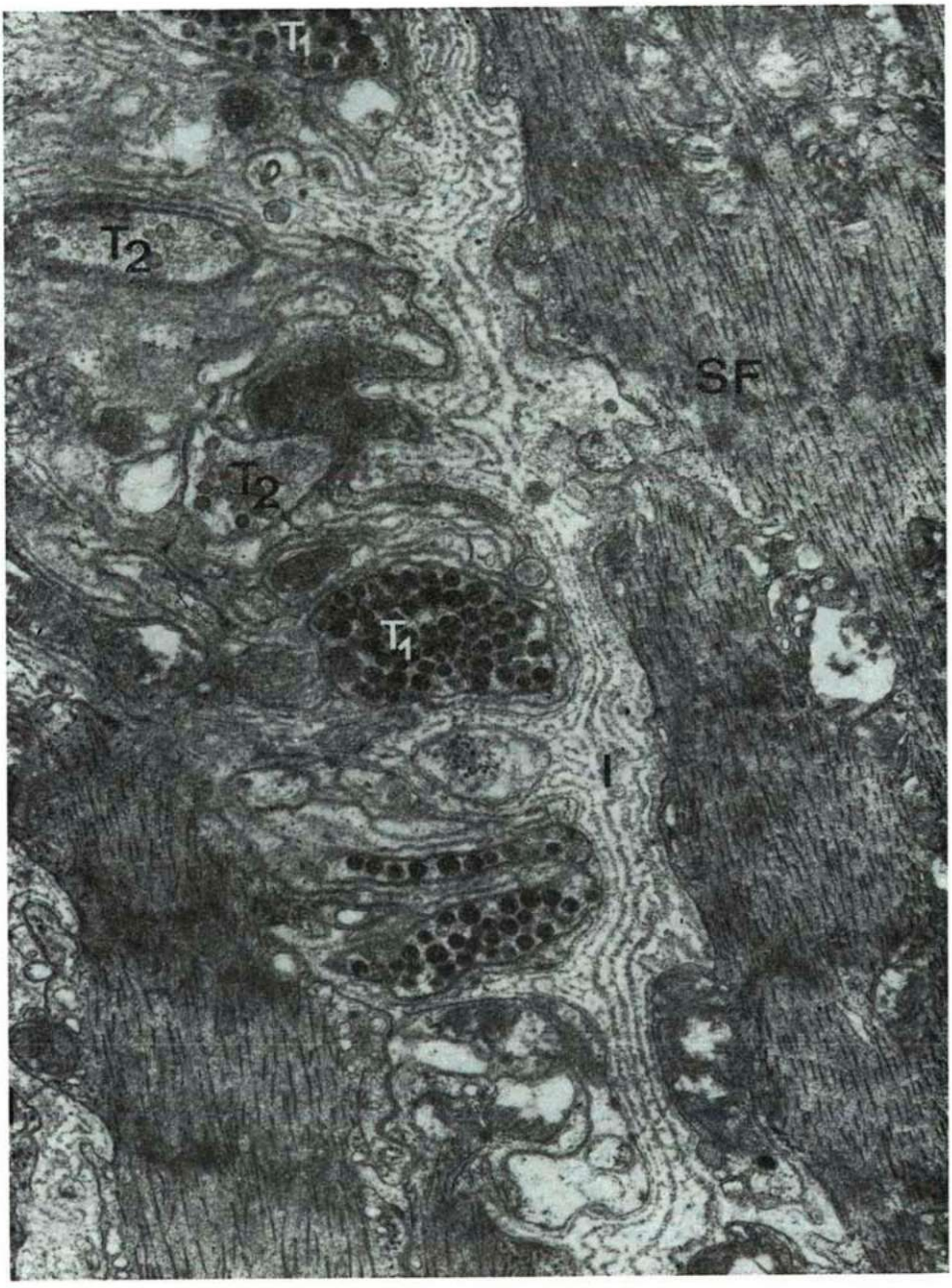


Fig. 6. Neurosecretory nerve fibres (T_1) among striated muscle fibres (SF) of *Locusta migratoria* gut. Note the synaptic terminals (T_2) with clear vesicles. I = interstitium x 16,000

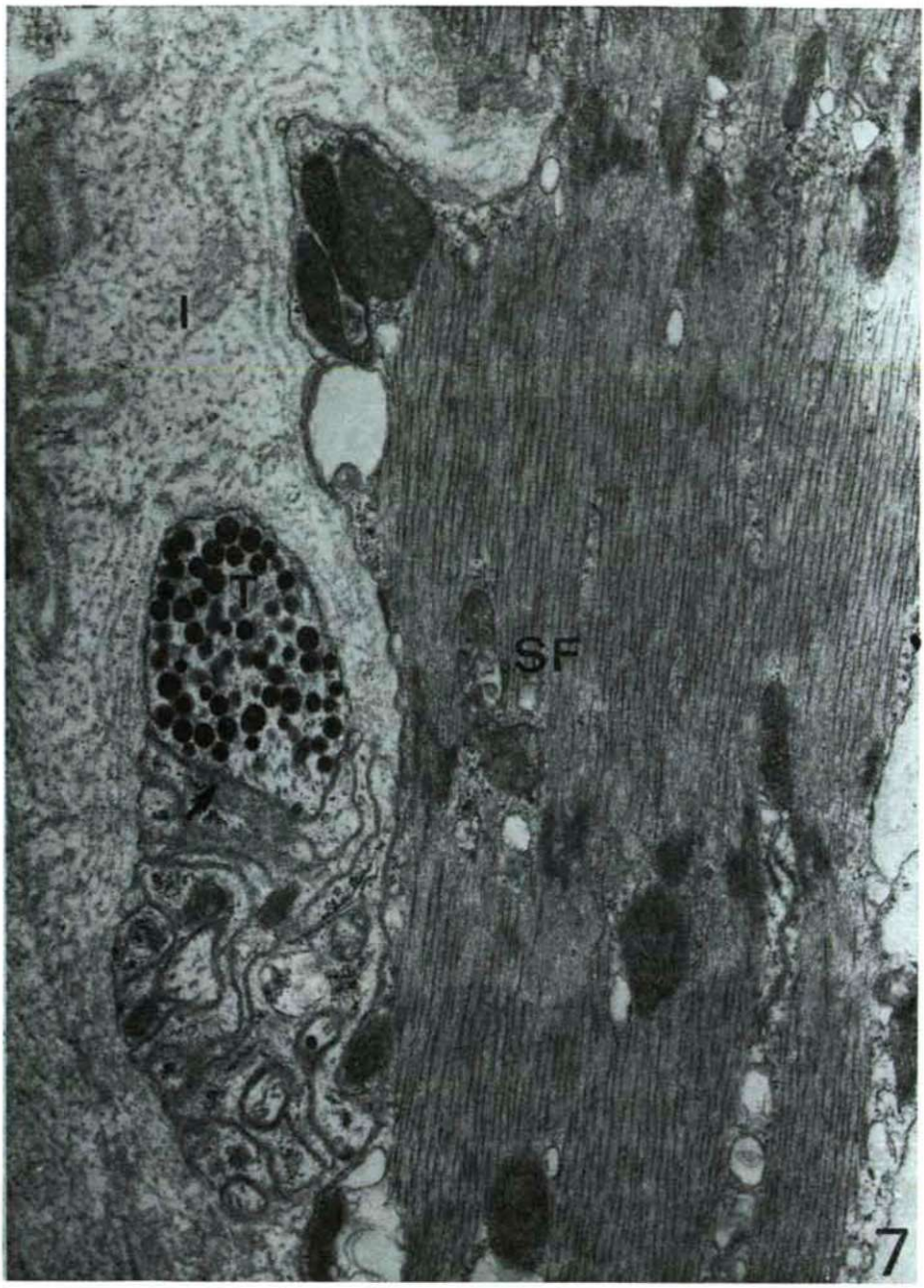


Fig. 7. Tight connection (arrow) between an axon terminal (T) filled with neurosecretory granules and a striated muscle process (SF) in the hindgut of *Locusta migratoria*. The membrane of the axon terminal is not surrounded by cytoplasmic process of glia cell, therefore, this is in direct contact with the interstitium (I). x 16,000

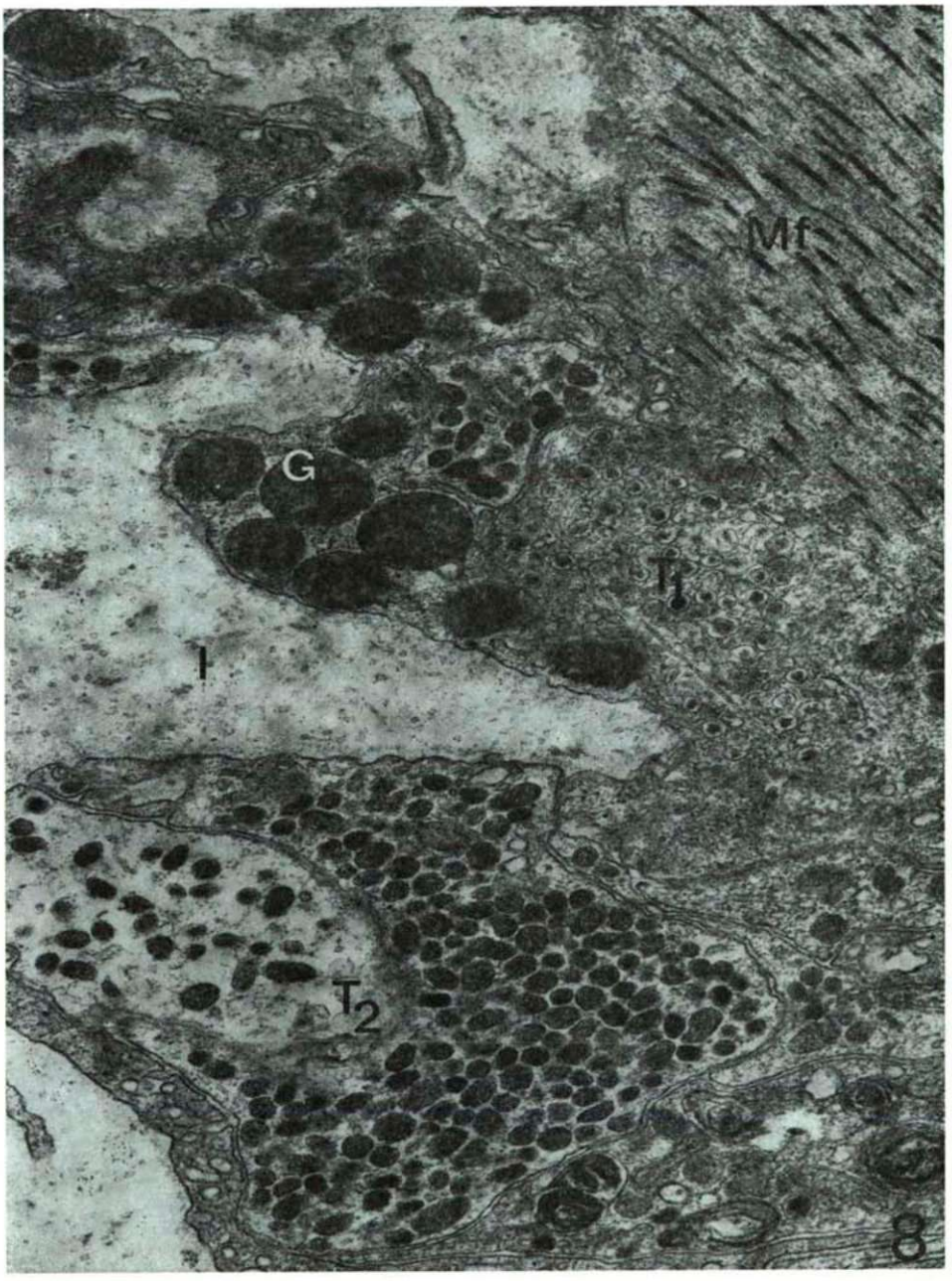


Fig. 8. Smooth muscle fibre (Mf) of snail gut being in tight morphological contact with nerve fibres containing dense-core vesicles (T_1) and those containing neurosecretory granules (T_2). G = glia cell process with granules. I = interstitium x 12,000

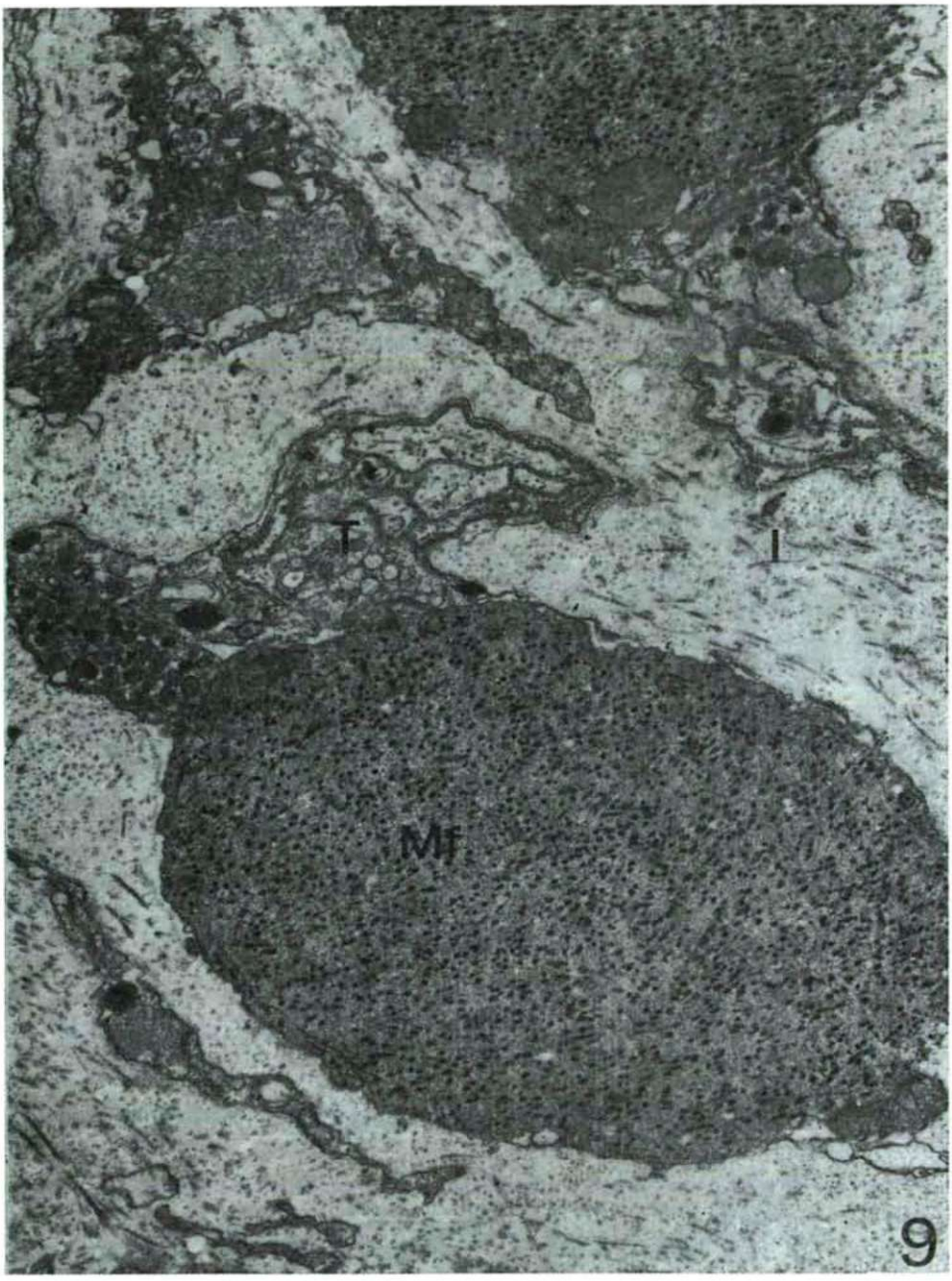


Fig. 9. Cross section of smooth muscle fibre (Mf). In its neighbourhood, note the nerve fibre (T) containing moderately electron dense granules with granular matrix. I=interstitium

x 18,000

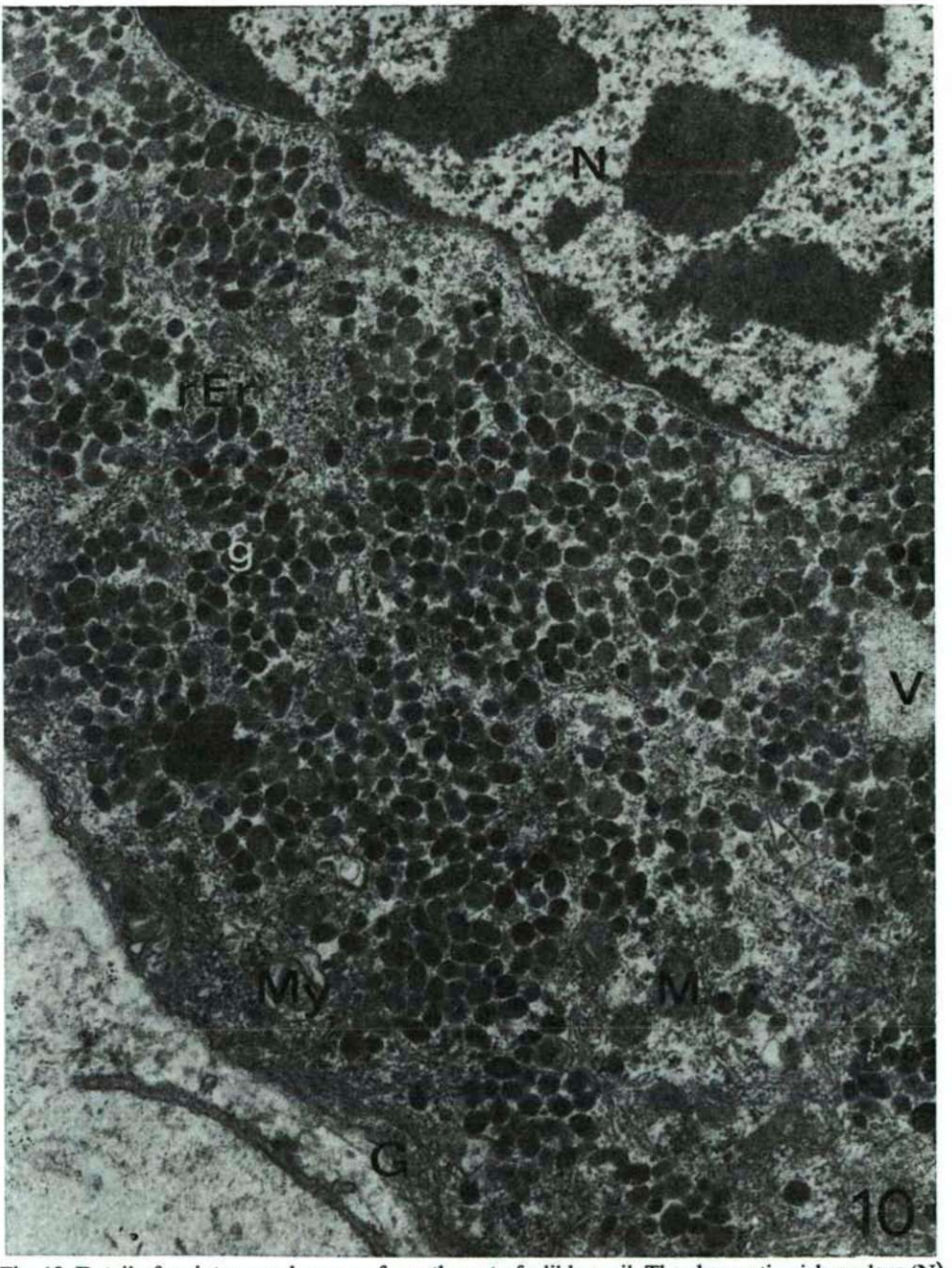


Fig. 10. Detail of an intramural neuron from the gut of edible snail. The chromatin-rich nucleus (N) is surrounded by cytoplasm packed with neurosecretory granules (g), large amounts of rough-surfaced endoplasmic reticulum (rEr) and mitochondria (M). The cell is covered by glial process (G). My = myeline figure, V = vacuole x 23,000

too, and even the accumulations of vesicles are detectable along the presynaptic membrane thickening (BENEDECZKY and MILLER, 1983). All these characteristic structural features refer to the fact that the functional characteristics of the striated muscular system of the insects gut differs in many respects (physiological behaviour, pharmacological sensitivity) from the common, classically-regarded physiological characteristics of smooth muscles. Surely, this is related to the characteristic habitude of insects, for example, the intensive mobility (see the long-range migration of *Locusta migratoria*), which has resulted in the development of a specific muscle system.

Regarding the other main characteristic difference, the presence of neurons in the snail and their absence in the insect, it is known that the gut muscles of the insects are supplied with central nerve fibres, viz. with nerves of the stomato-gastric nervous system in the area of the fore- and midgut, and with nerves coming from the ventral ganglions in the area of the hindgut (ANDERSON and COCHRANE, 1978). Neurons in the muscle layer of insect gut have only been reported by REINECKE et al., (1978), who found multinuclear, peripherally located neurosecretory neurons in the hindgut of *Manduca sexta* larvae. Comparing these cells with those described by us in *Helix pomatia,* we found essential differences: the neurosecretory cells in the insect gut are multinuclear with nuclei poor in chromatin, and the plasma is less abundant in neurosecretory granules than the neurosecretory cells of the snail. Investigations with physiological methods should elucidate the function of the neurons in the gut of insects and snail. On the basis of the heterogeneous ultrastructural picture of the neuromuscular junctions observed in the gut muscle of *Locusta migratoria* and *Helix pomatia,* it is presumable that various chemical substances may play a role as transmitter in the innervation of both striated and smooth muscles.

Several authors have attempted to elucidate the transmitters belonging to the various types of vesicles and granules in the AUERBACH's plexus of mammals (KOMURO et al., 1982, GORDON—WEEKS, 1981, 1982). The classification given in the survey of GABELLA (1979), suggesting that the synaptic, fibres containing clear vesicles are cholinergic, whereas those containing dense-core vesicles are aminergic has been queried by many others. GORDON—WEEKS (1982), e. g. demonstrated in experiments applying false transmitter (5-OHDA, 6-OHDA and 5,6-DHT) treatment that not every fibre containing dense-core vesicles can be regarded as noradrenergic, furthermore, that the apparent size and shape and the intactness of the vesicles greatly depend on the fixative. Therefore, the chemical nature of the transmitters cannot be judged merely on the basis of the morphological picture. It is also questionable to what extent the results of experiments carried out on mammals can be applied to invertebrates.

There is no doubt that the fibres containing large, highly electron dense granules — as we have demonstrated in the case of *Helix pomatia* — are the axons of one of the neurosecretory cells. The literature abounds in data suggesting that the large neurosecretory granules contain a neurosecretion of peptide-nature (RAABE, 1982). It is also supported by literary data that the neurosecretory granules leave the terminals through exocytosis (BENEDECZKY and MILLER, 1983) and exert their effect on the functioning of the muscule fibres. In the case of *Locusta migratoria,* it is also very likely that one of these neurohormones is identical with proctolin, a pentapeptide which was isolated from insect gut by BROWN and the occurrence of which in the

hindgut of the cockroach was demonstrated by ECKERT et al., (1981), using an immunocytochemical method.

The exact chemical structure of the transmitter substances of the snail neurosecretory fibres is still to be elucidated. Our assumptions, according to which the nerve terminals containing large dense-core vesicles may be aminergic, seem to be supported by biochemical data and our current histochemical studies (NEMCSÓK et al., FEKETE, personal communication).

References

ANDERSON, M. and COCHRANE, D. G. (1977): Studies on the midgut of the desert locust, *Schistocerca gregaria*. I. Morphology and electrophysiology of the muscle coat. — Physiol. Entomol. *2*, 247–253.

ANDERSON, M. and COCHRANE, D. G. (1978): Studies on the midgut of the desert locust *Schistocerca gregaria*. II. Ultrastructure of the muscle coat and its innervation. — J. Morph. *156*, 257–278.

ÁBRAHÁM, A. (1983): Ultrastructural studies on the gastrointestinal nervous system of *Helix pomatia*. — Acta Biol. Szeged, *29*, 1–2.

BENEDECZKY, T. and MILLER, T. A. (1983): Ultrastructure of the neuromuscular junction in the hindgut of *Periplaneta americana* and *Locusta migratoria*. — Z. mikrosk.-anat. Forsch. Leipzig, *97*, 505–519.

BROWN, B. E. (1975): Proctolin: a peptide transmitter candidate in insects. — J. Insect Physiol. *23*, 861–864.

ECKERT, M., AGRICOLA, H. and PENZLIN, H. (1981): Immunocytochemical identification of proctolin like immunoreactivity in the terminal ganglion and hindgut of the cockroach *Periplaneta americana* (L.). — Cell Tiss. Res. *217*, 633–645.

FAUSSONE PELLEGRINI, N. S. and CORTESINI, C. (1983): Some ultrastructural features of the muscular coat of human small intestine. — Acta Anat. *115*, 47–68.

GABELLA, G. (1979): Innervation of the gastrointestinal tract. — International review of cytology, *59*, 129–193.

GORDON-WEEKS, P. R. (1981): Are there noradrenergic synapses in Auerbach's plexus? — Scand. J. Gastrol. *16*, 181–182.

GORDON-WEEKS, P. R. (1982): Noradrenergic and non-noradrenergic nerves containing small vesicles in Auerbach's plexus of the guineapig: evidence against the presence of noradrenergic synapses. — Neurosci, *7*, 2925–2936.

KOMURO, T., BALUK, P. and BURNSTOCK, G. (1982): An ultrastructural study of nerve profiles in the myenteric plexus of the rabbit colon. — Neurosci. *7*, 295–305.

LLEWELLYN-SMITH, I. J., WILSON, A. J., FURNESS, J. B., COSTA, M. and RUSH, R. A. (1981): Ultrastructural identification of noradrenergic axons and their distribution within the enteric plexusses of the guinea-pig small intestine. — J. Neurocytol. *10*, 331–352.

RAABE, M. (1982): Insect neurohormones. — Plenum Press. New-York and London, 1–53.

REINECKE, J. P., GERST, J., GARA, B. O. and ADAMS, T. S. (1978): Innervation of hindgut muscle of larval *Manduca sexta* (L.) *(Lepidoptera: Sphingidae)* by a peripheral multinucleate neurosecretory neuron. — Int. J. Insect Morphol. Embryol. *7*, 435–453.

SALIMOVA, N. and FEHÉR, E. (1982): Innervation of the alimentary tract in chondrostean fish *(Acipenseridae)*. A histochemical microspectro-fluorimetric and ultrastructural study. — Acta Morphologica Acad. Sci. Hung. *30*, 213–222.

TÁNCZOS, J. és TÁNCZOS, J.-NÉ (1979): Fény- és elektronmikroszkópos vizsgálatok a *Helix pomatia* bélcsatorna falában levő idegvégződésekről. — (Light and electron microscopic studies on the nerve terminals of the gut muscle of *Helix pomatia*.) J. Gy. T-k. Főisk. Tud. Közl. *7*, 3–13.

Adress of the authors:
DR. K. HALASY
Department of Zoology
A. J. University
H-6701 Szeged P.O. Box. 659.
Hungary

Acta Biol. Szeged. 30, pp. 143–152 (1984)

COMPARATIVE MORPHOLOGICAL STUDIES ON THE ALIMENTARY CANAL OF *ISCHNURA ELEGANS* VANDERL. AND *LESTES VIRIDIS* VANDERL. *(ODONATA, ZYGOPTERA)* LARVAE

KATALIN HALASY and MÁRIA CSOKNYA

Attila József University, Department of Zoology, Szeged
Janus Pannonius University, Department of Zoology, Pécs

Received June 30, 1983)

Abstract

Macroscopic and microscopic observations concerning the alimentary canal of larvae of two *Zygoptera (Odonata)* species are reported. The histological layers of the trichotomous intestinal canal are muscle, epithelium and intima. The layers vary in thickness in the different sections of the intestine. The indentation observed in the gizzard as well as the regenerative and absorptive epithelial cell types of the midgut are noteworthy.

Key-words: Morphology of the gut in *Zygoptera* larvae.

Introduction

The majority of the studies on the anatomical, light- and electron microscopic structure of the intestinal canal of insects have dealt with species of health or agricultural importance. The epithelium of the midgut, its morphological and fine structural characteristics have been studied by BROWN (1980) in the tse-tse fly, by HECKER et al., (1971) in the malaria mosquito, by CIOFFI (1979) in *Manduca sexta* — an insect damaging tobacco — and by YU (1981) in the cabbage butterfly. Only few data are available on *Odonata* species, more exactly their larvae. ANDRIES (1970) has reported on the intestinal activity and regeneration of the *Anisoptera Aeschna cyanea* during the digestive cycle and MOENS (1980) on the hindgut of larvea of a few *Anisoptera* species.

In the course of examinations regarding the respiration and osmoregulation of dragonfly larvae it became justified to obtain exact knowledge of the distinct parts and structure of their digestive mechanism since the mentioned functions are in relationship with certain sections of the digestive system (BODINE, 1918; CAROLL, 1918; CIOFFI, 1979; KOCIAM, 1930; KOMNICK, 1977; LEE, 1929; MOENS, 1980; TILLYARD, 1917). As our previous studies were carried out on *Zygoptera* species we chose *Ischnura elegans* VANDERL. and *Lestes viridis* VANDERL. for these investigations too.

Material and methods

Specimens in the last larval stage, approximately equal to size, 21–22 mm in length including the caudal branchiae were examined. The larvae were collected from small deadwaters near Szeged in May, 1981. For light microscopic histology the material was fixed in Bouin's and Carnoy's fixative and in 10% neutral formalin then embedded in paraffin. 5–7 μm sections were stained with haemateineosine or according to Mallory.

For electron microscopy the material was fixed in Karnovsky's fixative for 4 h at +4 °C then treated at room temperature with 2% osmium tetroxide (pH: 7,2) dissolved in Millonig's buffer. After dehydration in an ascending alcohol series the material was contrasted overnight with uranyl acetate dissolved in 70% ethanol and it was embedded in Durcupan ACM synthetic resin. The ultrathin sections were prepared with Reichert ultramicrotome and recontrasted with lead citrate. The sections were examined and photographs were taken in a JEOL 100B electron microscope.

Of the numerical data referring to the lenght of the certain parts of the intestine each represents the average of the results measured on 10 larvae being of the same degree of maturity.

Results and discussion

The alimentary canal of the studied *Zygoptera* larvae is a straight tube extending from the pharynx in the head till the anus in the last abdominal segment; it doesn't penetrate the caudal gills. All parts of the intestine characteristic of the Arthropods in general are to be found in dragonfly larvae (ARA, 1976; BEHURA et al., 1974; MACGOWN et al., 1981; MAKHDOOMI, 1977; QUARTEY et al. 1973).

The stomodeum is the intestinal section reaching from the head to the 2nd or 3rd abdominal segment. It is gradually dilating backwards and suddenly narrowing by its end. It consists of the pharynx, the oesophagus, the ingluvies and the gizzard

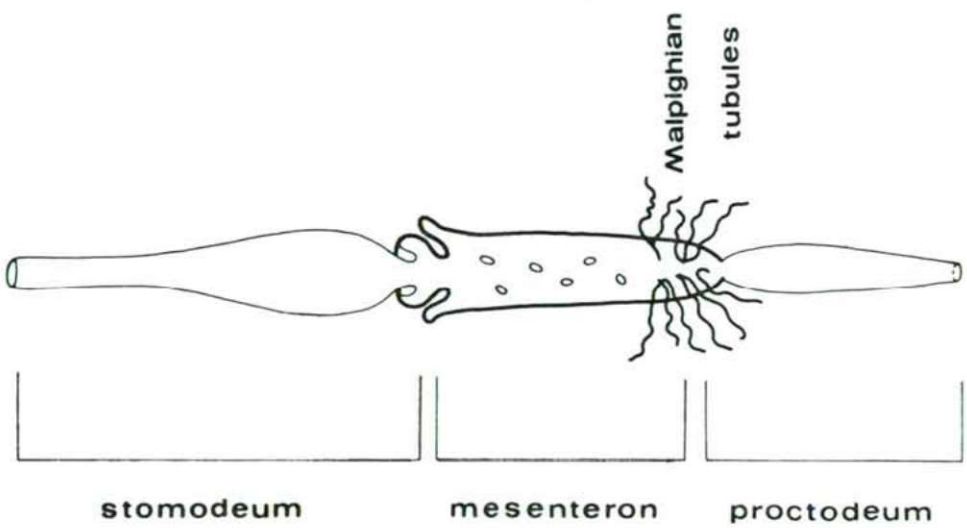


Table 1. Tripartition of the alimentary canal of the *Zygoptera* larvae.
Table 2.:

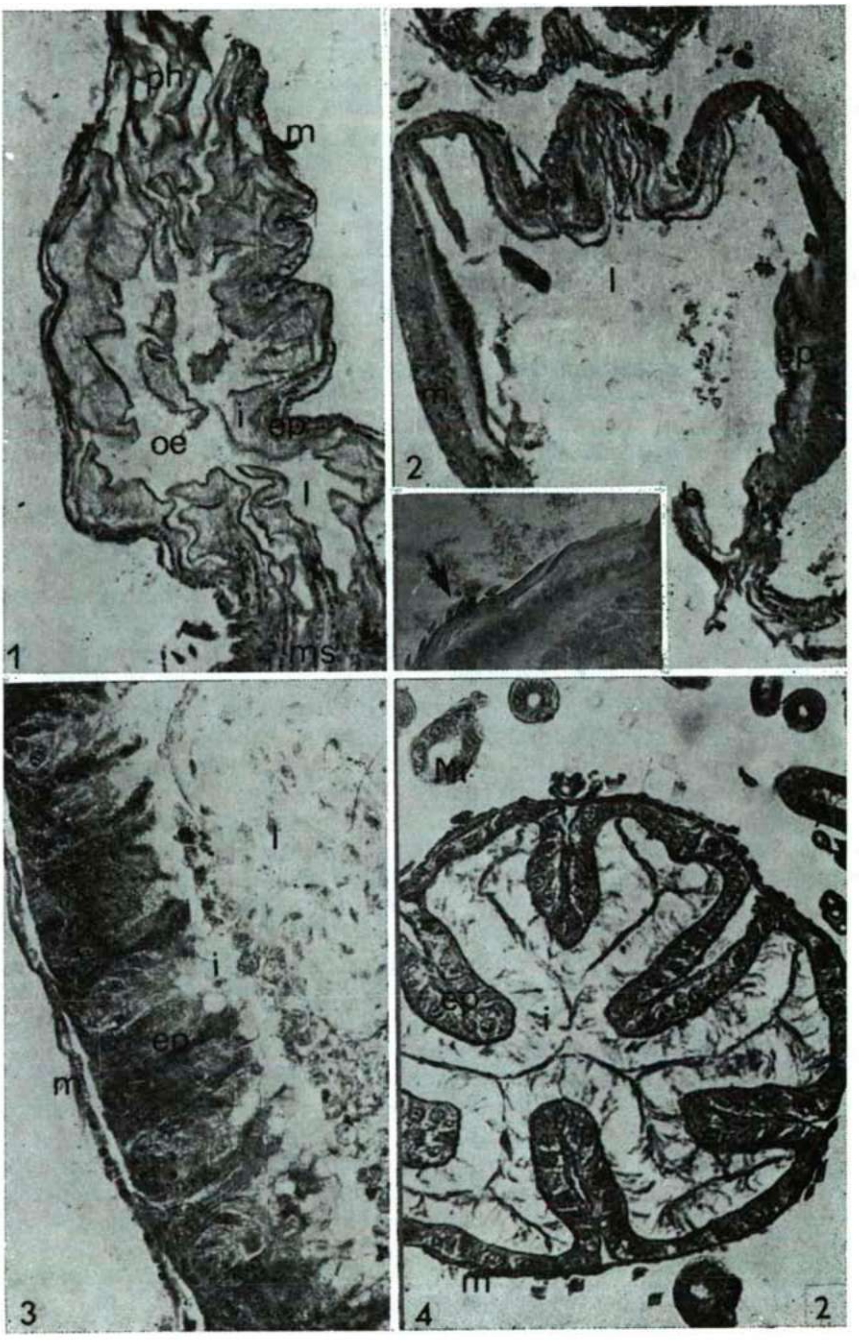
Plate 1. Longitudinal section of *Lestes viridis* foregut with longitudinal plication. ph=pharynx; oe=oesophagus; Ms=entry of gizzard; m=muscle; ep=epithelium; i=intima l=lumen. Haematein-eosin stain. x 150

Plate 2. Longitudinal section of *Ischunra elegans* grinding stomach (gizzard). The entry of the stomach can be seen in the upper part of the picture and below the valves terminating towards the midgut (b). On the insert the arrow shows the grinding indentation. m=muscle; ep=epithelium; i=intima; l=lumen. Haematein-eosin stain. x 150 (insert: x 200)

Plate 3. Longitudinal section of the midgut wall of *Lestes viridis* with dark and light cells. m=muscle; ep=epithelium; i=intima; l=lumen; Haematein-eosin stain. x 200.

Plate 4. In the cross-section picture of the *Ischnura elegans* hindgut the epithelium (ep) forms 6 longitudinal plicae. m=muscle; i=intima; Mt=Malpighian tubules. Haematein-eosin stain. x 200.

Table 2.



(Table 1). This applies both species studied by us and the corresponding parts of the intestine appear identical in shape and size. The pharynx, about 2 mm long, is a strongly dilating tube situated in its whole length in the head. From there it suddenly widens to the double or triple and continues in a section of spindle form (length, 3,5—4 mm) along the thorax. Even when filled with aliment a longitudinal plication is detectable (Table 2, Plate 1.). which is more striking in *Ischnura elegans* than in *Lestes viridis*. The aliment is stored for a considerable period near the end of this intestinal section since, it cannot reach the following section but at determined intervals. Due to this role of storage this part of the oesophagus is called ingluvies (TILLYARD, 1917; WHEDON, 1918).

The thickest and strongest part of the foregut the gizzard is in the abdomen (Table 2. Plate 2.) Its size and location somewhat differs in the two species and in *Ischnura elegans* there are differences between the sexes. In the female individuals of *Ischnura elegans* the gizzard being 0,5—0,8 mm long ends in the 2nd abdominal segment, while in the male individuals it is larger ending in the 3rd abdominal segment. There are no sex differences regarding *Lestes viridis*. Similar data have been reported by others: for example in *Calopteryx* species the location of the gizzard differs in the two sexes however in *Lestes unguiculatus* the gizzard is of the same size and location in male and female individuals (TILLYARD, 1917; WHEDON, 1918). Valves and strong plication can be seen at both the inlet and the outlet of the gizzard, particularly well in histological sections (Table 2. Plate 2.).

The mesenteron or midgut is to be found in the abdominal segments 3—6, or 4—6, being a 3—3,5 mm long section uniform in structure. The Malpighian tubes can be observed at its end parts (Table 1).

The anatomical relations of the hindgut are the most controversial. TILLYARD (1917) described the hindgut of various *Zygoptera* larvae as being uniform in structure while others (BODINE, 1918; CAROLL, 1918; CULLEN, 1918; KOCIAM, 1930; KOMNICK, 1977; LEE, 1929) found different sections in it. It the larvae studied by us the hindgut could be divided into two parts: the frontal ileum being tubular in shape (3 mm), and the vestibule (0,5 mm) ending with the anus.

Histologically the alimentary canal can be divided into 3 layers, such as the outer muscular layer followed by the epithelial layer on the basal lamina and then the innermost intima which is the product of the epithelial cells (Table 2. Plates 1—4.). These layers are found along the whole intestine however they may become modified according to the functions of certain intestinal sections. The greatest variation is shown by the epithelial layer which is built up of cell indviduals along the alimentary canal. In the foregut the cells are flat in the area of the pharynx., oesophagus and ingluvies with a homogenous intima (cuticula), a layer ensuring the protection of epithelial cells (Table 2. Plate 1.) On the base of the cells a structureless thin basal lamina and a similarly thin muscular layer can be distinguished. The separation of the oesophagus and ingluvies seems groundless on the basis of the histological sections. Their structure is alike and they have the same function: storage of nutriments.

The gizzard differs in structure from the other foregut sections (Table 2. Plate 2). The epithelial layer is made up cuboid epithelial cells and the intima covering the surface differs in the two species. In *Ischnura elegans* there is an indentation in the centre of the gizzard arranged in lo layers. In *Lestes viridis* such an indentation was not detected. These teeth — together with the rather thick muscle layer — presumably assist the mincing of the aliment.

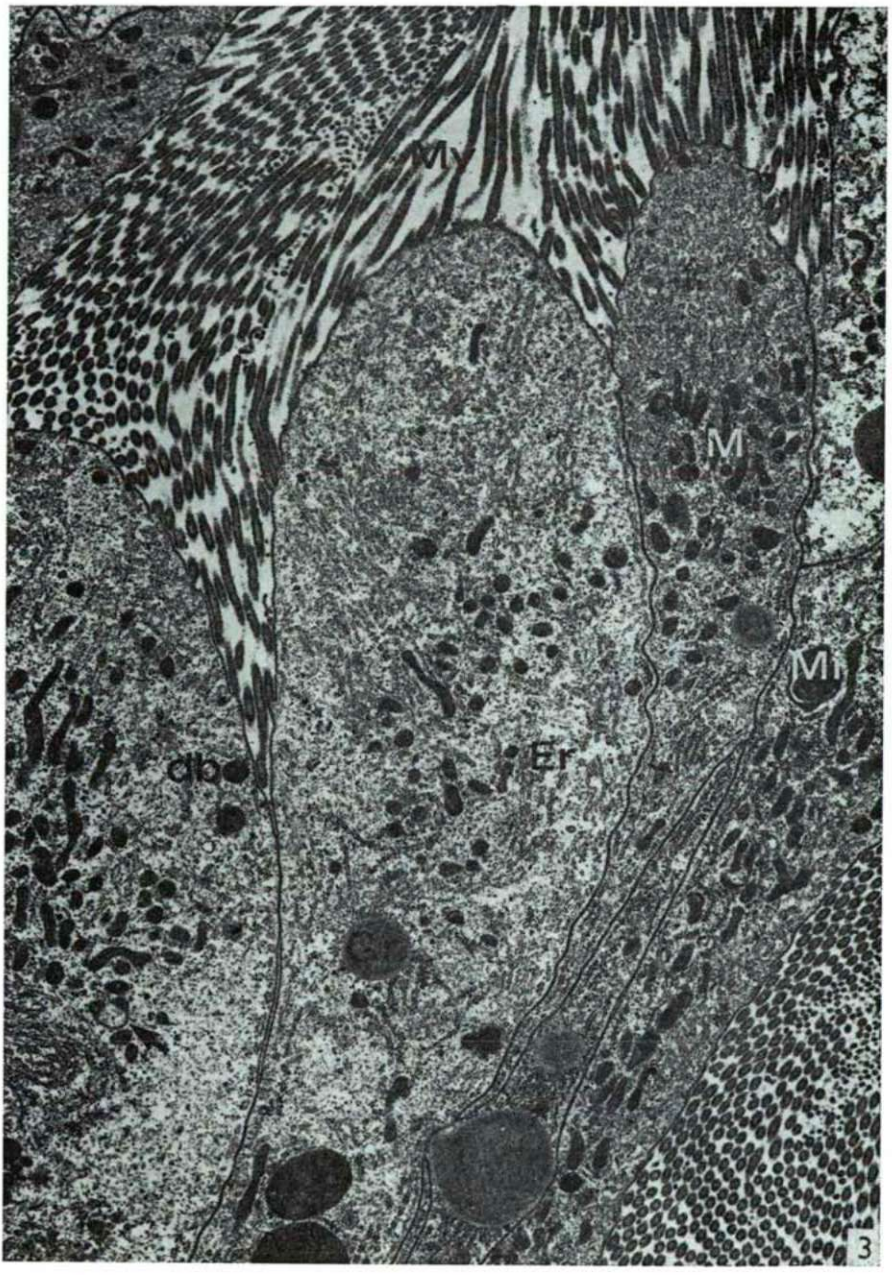


Table 3. Absortive epithelial cells of the midgut of *Ischnura elegans*. Apical section with microvilli (Mv). Er = endoplasmic reticulum; M = mitochondria; Mi = myeline figure; db = dense body; Gr = granule. The arrow indicates a mitochondrion of peculiar shape. x 7700.

Valves and extremely developed plicae are to be found at the inlet and outlet of the stomach. The plicae are formed by the oesophagus at the fore-part of the stomach and by the gizzard itself at the end part. The right and the left valve plicae are identical in appearance therefore these seem to regulate the proceeding of a determined amount of aliment, less probably the direction of progress (KAUSHIK, 1969).

The epithelial cells of the midgut vary in staining with haematein-eosin (Table 2. Plate 3.). Some of the cells stain densely and are elongated, cylindrical, the others show paler staining and are flatter. Latter form small groups among the dense cells. The pale so-called regenerative cells are small having large nuclei. They occur in particularly large numbers in the caudal section of the midgut, similarly as in that of the *Anisoptera* larvae (ANDRIES, 1970). Electron microscopically, the cylindrical epithelial cells of the midgut show the characteristic appearance of absorptive cells (Table 3). There are many elongated microvilli on their apical surface with dense bodies close to their stem. Mitochondria, rough-surfaced endoplasmic reticulum and lipid granules are seen in the cell plasma. The mitochondria are occasionally vacuolized or are of characteristic form resembling telephone receiver (Table 3. arrow). Multivesicular bodies (Table 4. Plate 1.), large (diameters of 100–150 nm without coat, and the double with coat) coated vesicles having particular structure (Table 4. Plate 2). furthermore rather high amounts of various-sized lipid droplets (Table 4. Plate 3.) can be found in the central region of the epithelial cells. Nearing the basal lamina we are aware of light-microscopically distinguishable regenerative cells among the absorptive ones. The regenerative cells have large nuclei rich in chromatin and narrow perinuclear cytoplasmic rim (Table 5).

The fundamental histological layers are also present in the hindgut: the outer muscular layer then the cylindrical epithelial layer on the basal lamina with rather thick cuticle on their surface facing to the lumen. These layers can well be distinguished in both the haematein-eosin stained sections (Table 2. Plate 4) and the electron micrographs (Table 6). At high magnification basal invaginations are seen on the basis of the cells. Rather contradictory data have been reported regarding the function of the hindgut: according to BODINE (1918) and CAROLL (1918) the rectum serves the function of respiration — tough it doesn't form respiratory chamber in these larvae and it functions in similar manner as the tracheal gills. This assumption is doubted by several authors since it is not supported by epithelial cells supplied with tracheas (KROGH, 1941); on the other hand no relationship can be demonstrated between the frequency of the ileal contractions and the oxygen content of the surrounding water (PENNAK et al., 1944). CIOFFI (1979) and KOMNICK (1977) suggest that considering the lack of ion-absorption areas on the body surface of *Odonata* larvae, osmoregulatory functions are served by this area of the hindgut in *Zygoptera* larvae. These authors designated the high cylindrical epithelial cells of the ileum as chloride epithelia and they have proved their assumptions experimentally. On the basis of the obtained morphological picture we accept this latter up-to-date viewpoint.

Table 4.:

Plate 1. Multivesicular body (Mb) in the absorptive epithelial cell of *Ischnura* midgut. Gr = lipid granule. x 34,100.

Plate 2. Coated vesicle (Cv) in the absorptive epithelial cell of *Ischnura* midgut. M = Mitochondrion. x 47,500

Plate 3. Medial area of the epithelium cells of the *Ischunra* midgut rich in lipid granules (Gr). Er = endoplasmic reticulum. x 16,400.

Table 4.

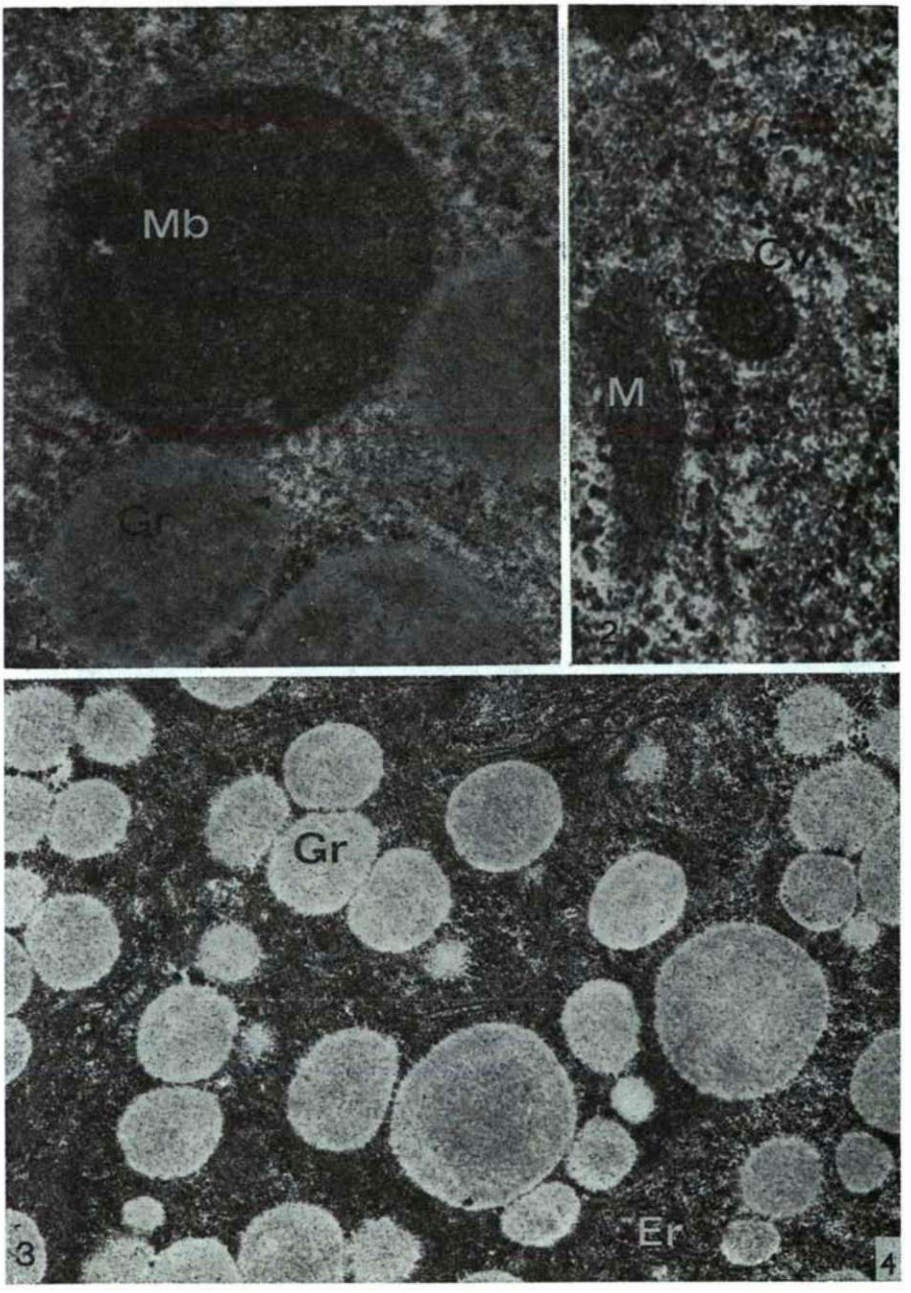


Table 5. Regenerative cells among absorptive epithelial cells on the basal lamina (Bl). N = nucleus; Bi = basal invaginations. x 9,700.

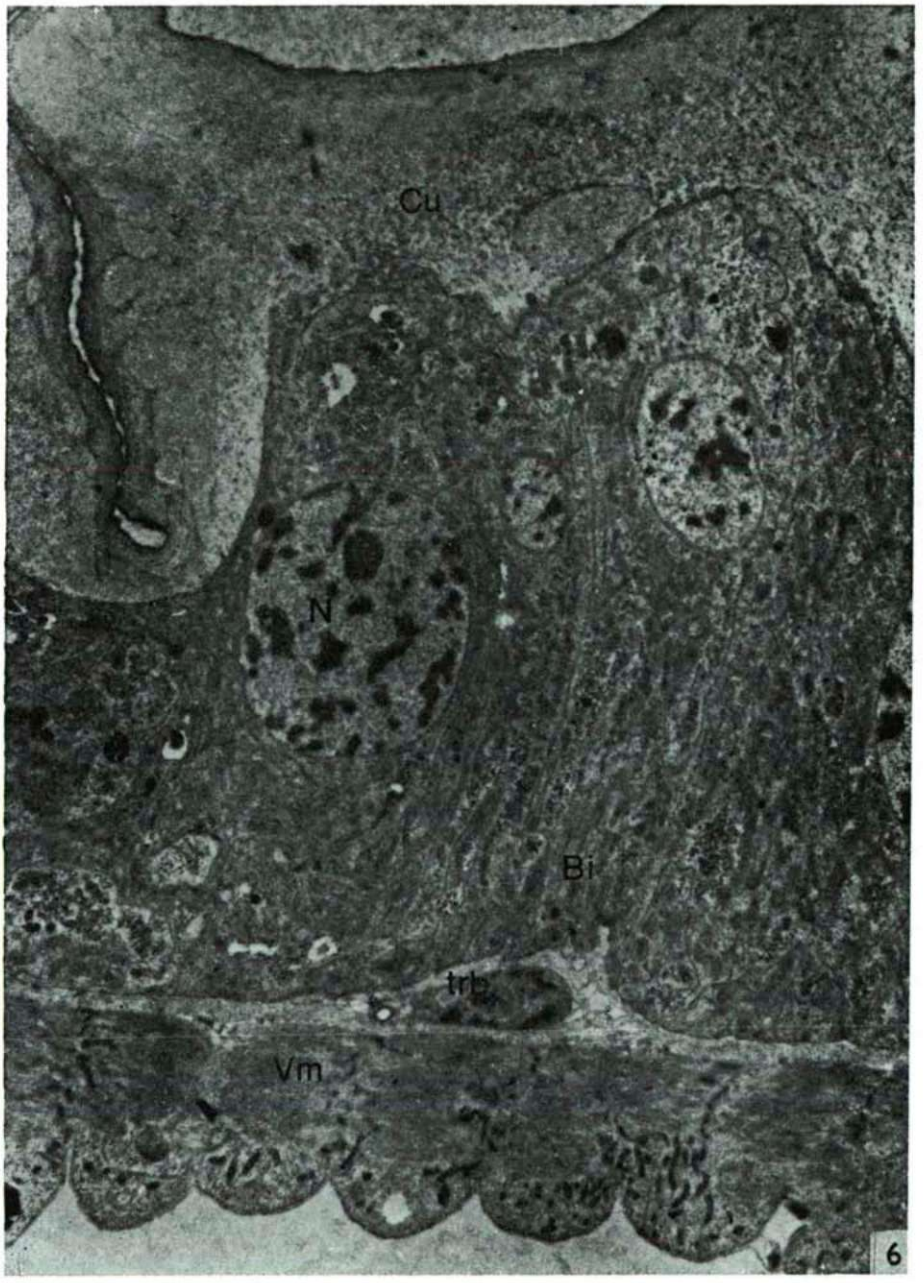


Table 6. Detail from the hindgut of *Ischnura elegans*. The thick cuticle (Cu) is of lamellar structure above the cylindrical epithelial cells. N = nucleus; Bi = basal invagination; trb = tracheoblast; Vm = visceral muscle. x 4,000.

References

ANDRIES, J. C. (1970): Activité des nids de régénération de l'intestin moyen de la larva d'*Aeschna cyanea* au cours d'un cycle digestif. — J. Insect Physiol. *16*. 1961—1973.

ARA, P. (1976): Gross morphology of the digestive tract of some Cecidomyides. — Gecid. Indica *11*, 57—67.

BEHURA, K. B., DASH, M. M. and BEHERA, K. S. (1974): On the morphology and histology of the digestive system of the giant water bug, *Belostoma indicum* Lep. and Serv. *(Belostomidae, Heteroptera)*. — Prakrnti-Utral Univ. J. Sci. *11*, 11—28.

BODINE, J. H. (1918): Experimental results in *Ischnura* and *Enallagma*. — Proc. Acad. Nat. Sci. Phila. *70*, 103—113.

BROWN, R. P. (1980): Ultrastructure and function of midgut epithelium in the tsetse *Glossina morsitans* Westw. *(Diptera: Glossinidae)*. — J. ent. Soc. sth. Afr. *43*, 195—214.

CAROLL, M. (1918): The hindgut, abdominal tracheae and rectal respiration in the larva of *Mecistogaster modestus* from Costa Rica. — Proc. Acad. Nat. Sci. Phila. *70*, 86—103.

CIOFFI, M. (1979): The morphology and fine structure of the larval midgut of a moth *(Manduca sexta)* in relation to active ion transport. — Tissue and Cell *11*, 467—479.

CULLEN, A. M. (1918): Rectal tracheation of *Argia putrida* larva. — Proc. Acad. Nat. Sci. Phila *70*, 75—81.

HECKER, H., FREYVOGEL, T. A., BRIEGE, H., and STEIGER, R. (1971): Ultrastructural differentation of the midgut epithelium in female *Aedes aegypti* (L.) *(Insecta, Diptera)* imagines. — Acta Trop. *28*, 80—104.

HOUK, E. J. (1977): Midgut ultrastructure of *Culex tarsalis (Diptera: Culicidae)* before and after a blood-meal. — Tissue and Cell *9*, 103—118.

KAUSHIK, S. C, (1969): On the morphology of the giant water bug, *Belostoma indicum* Lep. and Serv. *(Heteroptera: Belostomatidae)*. Part IV. Morphology and histology of digestive system — J. Anim. Morphol. Physiol. *16*. 1—16.

KOCIAM, V. (1930): O respiracnim vyznamu tracheálnich lupénku u Agrionu. — Publ. Fac. Sci.Univ. Charles *108*, 1—40.

KOMNICK, H. (1977): Chloride cells and chloride epithelia of aquatic insects. — Int. Rev. Cytol. *49*, 285—329.

KROGH, A. (1941): A comparative physiology of respiratory mechanism. — Univ. Press, Pennsylvania.

LEE, M. O. (1929): Respiration in the insects. — Quart. Rev. Biol. *4*, 213—232.

MACGOWN, M. W. and SIKOROWSKI, P. P. (1981): Digestive anatomy of the adult boll weevil, *Anthonomus grandis grandis* Boheman *(Coleoptera: Curculionidae)*. — Ann. Ent. Soc. Am. *74*, 117—126.

MAKHDOOMI, S. M. A. (1977): Comparative study of the alimentary canal in the larvae and adults of *Syrphus balteatus* Degeer. — J. Agric. Res. *2*, 110—121.

MOENS, J. (1980): Microscopic and ultrastructural study of different epithelial elements in the ileum of larvae of Anisopteran dragonflies. — Odonatol, *8*, 309—315.

PENNAK, R. W. and MCCOLL, C. M. (1944): An experimental study of oxygen absorption in some dragonfly naiads. — J. Cell. comp. physiol. *23*, 1—10.

QUARTEY, S. Q. and KUMAR, R. (1973): Structure of the alimentary canal and reproductive organs of some adult *Neuroptera*. — Ent. scand. *4*, 91—99.

TILLYARD, R. J. (1917): The biology of the dragonflies. — Cambridge Univ. Press Cambridge, England.

WHEDON, A. D. (1918): The comparative morphology and possible adaptations of the abdomen in the Odonata. — Trans. Amer. Ent. Soc. *44*, 373—437.

YU, C. H., KIM, W. K. and KIM, C. W. (1980): Secretory granular cells in the midgut epithelium of the *Blattella germanica* L. — Kor. J. Electr. Micr. *10*, 27—32.

YU, C. H. (1981): Morphology of the midgut epithelial cells in the cabbage butterfly *Pieris rapae* L. — Bull. I.B.S. Inha Univ. *2*, 93—99.

Adress of the authors:
DR. KATALIN HALASY
DR. MÁRIA CSOKNYA
Department of Zoology
A. J. University
H-6701 Szeged, P.O. Box 659
Hungary

Acta Biol. Szeged. 30. pp. 153–158 (1984)

CHARACTERISTICS OF THE POPULATION OF AN *ISOPODA* SPECIES (*TRACHELIPUS NODULOSUS* C. L. KOCH) AT SANDY SOIL GRASSLAND

ERZSÉBET HORNUNG

Attila József University, Department of Zoology, Szeged

(Received October 25, 1983)

Abstract

During the course of studies performed between 1979–1982 on the population of a *Trachelipus nodulosus* C. L. KOCH *(Isopoda)* species living at sandy soil grassland, it had been determined that the dispersion of the species shows clumping dispersion type at the area. Its habitat is only limited to more humid, deeper sites developed by wind. Investigations on the dynamics of the population revealed that the factors of microclimate being effective through the local microclimate relations do not only determine the distribution of the population, but also have influence on its volume, seasonal changes, as well as the activity of the species.

Key-words: Isopoda, sandy soil grassland, dispersion, population dynamics, reproductive period, fecundity

Introduction

The humidity of the air is commonly known to be a key factor in the respect to the existence of the *Isopoda.* Therefore, it is of particular interest to study their occurrence under extremely dry conditions. A possibility for this was given at the region of the Kiskunság National Park, within the research activities of the ecological group of the Department of Zoology, Attila József University. The studies have been being carried out since 1976, at a model area of 2 ha withdrawn from grazing (MÓCZÁR et al., 1980). The long-lasting continuous study makes possible among others the revelation of the population characteristics of various animal species. There are no antecedants of such nature in Hungary regarding investigations on *Isopoda.* Similar studies were carried out in America (PARIS and PITELKA, 1962, SORENSEN and BURKETT, 1977) at grass-plots concerning the *Armadillidium vulgare* species, and in Japan (SAITO, 1965) at forests on the *Ligidium japonicum.*

The single Isopoda species, the *Trachelipus nodulosus,* living at the studied area plays role in the decomposing chain, the productivity parameters of which had been investigated earlier, under laboratory conditions (HORNUNG, 1979, 1981a, b). The species itself is xerophyll, and occurs at open, more sunny areas.

The studied area

The pedological characteristics, macro- and microclimatic relations, main plant- and animal-communities, their production, etc. of the studied area located at the Bugac district of the Kiskunság National Park are known from the works of BODROGKÖZY and FARKAS, (1981), KÖRMÖCZI et al., (1981), MÓCZÁR et al., (1980). The extreme relations of sandy plains are characteristic of the micro-

climate: semiarid climate, drought-hazardous summer, insufficient precipitation supply in summer, great daily and annual fluctation in temperature, dry sandy soil. All these would exclude the existence of the *Isopoda*. The geographic structure of the area is the cause for the fact that under the mentioned circumstances they nevertheless turn up and their population is capable of maintenance. The surface of the region is distributed by wind-furrows. with grade differences of 1–2 m as the consequence of which the area forms a mosaic complex: varying plant associations and microclimate relations develop, also affecting the quatily and quantity distribution of the animals living there.

From the viewpoint of the *Isopoda*, the wind-furrows have distinct significance since these animals requiring high humidity find their vital conditions here. The characteristic plant community of the wind-furrows is the *Molinio-Salicetum rosmarinifoliae*. Here, the plant covering is great (85–100%), the plants are high (50–60 cm), and this is the area where the underground water content reaches the maximum. The humidity decreases at later time-point in the mornings and increases earlier at sunset than at the neighbouring areas of higher level. The temperature also remains lower here by 3–4 °C (KÖRMÖCZI et al., 1981).

Methods

The Isopoda were collected with Barber type ethylene-glycol pit fall traps, of which two variants were applied:

For the collection of the epigeous, surface-mobile animals the traps were placed in groups of 5, at the points of a 50×50 cm sized square and at the intersection of its diagonals, resp. The averages of the data regarding these trap groups were applied as samples. 12 such trap groups were placed at the area.

The other type was the variant of the Barber trap placed in the soil, above which a glass-plate was layed and the replaced piece of grass, resp. This type enables the collection of the mobile animals having their living place in the root zone, in the upper 10–20 cm of the soil. The largest amount of *Isopoda* could be collected here, as these pits serve as excellent shelter for the hygrophylic animals.

The traps were emptied bi-weekly or monthly and the caught animals were kept in 70% ethanol, making possible their further laboratory processing. Their body lengths were then measured under microscope, the sex determined, and the eggs, embryos found in the brood pouch of the pregnant females; and/or the juvenile individuals were counted.

Results and their evaluation

The material of the above described two kinds of traps made possible two different modes of approaching the Isopod population at the studies area. As mentioned in the foregoings, the trap groups of 5 concern every habitat type of the area, thus on the basis of the obtained material conclusions could be drawn regarding the dispersion ot the *Isopoda* at the whole of the area. On the other hand, being familiar with the characteristic feature of the *Isopoda*, according to which they move little under optimal environmental conditions (WHITE, 1968) — that is, their activity of movement increases with the temperature rise of the environment, at the time of decrease in relative humidity (RH); i. e. when the circumstances become drier — the data of these traps reflect the activity of these animals and its seasonal changes. It could also be determined on the basis of the data obtained from the traps that the *Isopoda* aggreagate at the wind-furrows, where the unfavorable physical conditions of necessity (mainly the lack of water) are compensated by the somewhat more humid microclimate. Therefore, the species shows clumping dispersion at the area (HORNUNG, 1979).

The graphs of Figure 1/a demonstrate the data of the trap groups of 5 in the years 1979–1982. ($\sum N$ = total individual number of samples). It can be seen from these that the course of the curves for the certain years is close to similar. The maxi-

mal values are reached in the 7th-8th months. The almost similar course of the curves, as well as the concurrence of the peaks would allow to draw the conclusion that the autoregulating factors within the Isopoda population compel the maintenance of the population volume at a constant level — within certain limits. In the present case this is not so; the autoregulation is only apparent. If being periodically recurrent, external factors may also cause periodically repetitive activity peaks. Here the population's volume and annual dynamism, their periodicity are also determined by external environmental — firstly climatic — factors.

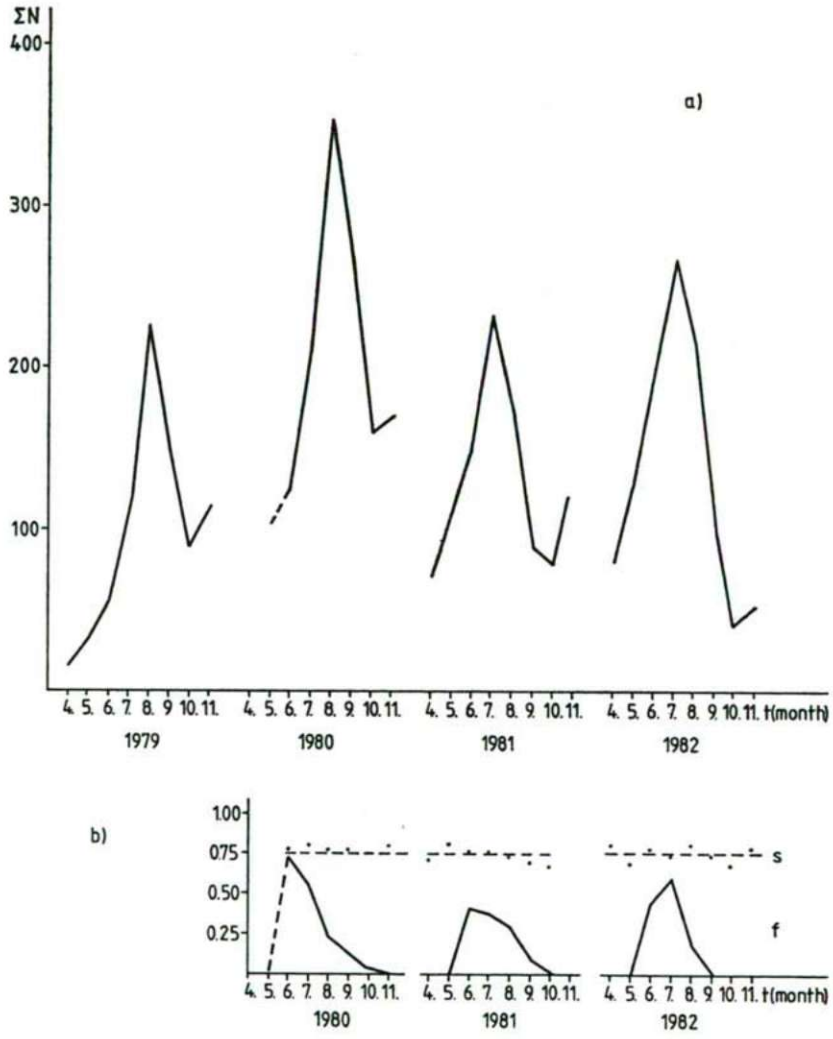


Fig. 1.a. Changes in the total individual number (ΣN) of the *Trachelipus nodulosus* species for the years 1979–1982.
Fig. 1.b. Changes in the values of the species' sexual rate (s) and fecundity (f) in 1980–1982.

It can be observed from the Figure that the data of the year 1980 are essentially higher than those of the rest. The explanation to this is presumably found in the fact that the above mentioned year was relatively more rainy (3–4 folds that of the previous year). Through the Rh positive changes in the soil moisture and vegetation this higher amount of precipitation also showed favourable influence on the population dynamics of the *Isopoda*. In such cases there is an increase in the number of those habitats, and size of their patches, resp., where the *Isopoda* find their conditions for living; their mortality presumably becomes lower, and their reproduction shows more favourable tendency, resp.

Figure 1/b demonstrates the changes in the sexual rate and fecundity of the studied population regarding the years 1980–1982. It can be seen that while the sexual rate (s) is close to constant not only within a year, but even between the certain years, the fecundity (f) — i. e. the ratio of the pregnant females — shows a rather divergent development. The fecundity of the previously emphasized more rainy year of 1980 is the highest. The value of the sexual rate is around 0.75, which means that about 75% of the collected individuals are females, differing from the values reported on other populations of the *Armadillidium vulgare* by PARIS and PITELKA (1962), and of the *Trachelipus nodulosus* by HORNUNG (1979).

Considering the values of fecundity, it is also proved that the number of offspring increases on the effect of more favourable climatic factors. The number of offspring may even increase with the larger amount of eggs per females, which in the case of the studied population reached the value of 14–40 eggs/females. The eggs/number of offspring per females show linear increase with the growth of the size of the females (HORNUNG, 1979). Author found even essentially higher values in the case of another population of the same species, living under less extreme circumstances: 32–75 eggs/females (HORNUNG, 1979).

The annual dynamism of the population can be followed on the basis of the data regarding the other applied trap type. Studying the year 1980, the population curve previously discussed (Fig. 1/a) can further be improved on the basis of the bi-weekly collections. Figure 2 demonstrates two maximums for the curve, the explanation to which is as follows: the studied T. nodulosus species has its reproduction period from the middle of May to the beginning of September, and under our climatic relations it takes place in two larger periods; one having its peak in May–June, the other in July–August. Since the *Isopoda* females carry their eggs, later their larvae in their marsupium till complete development (cc. 1 month), the first juvenile individuals appear at the end of June, and the last at the beginning of September. PARIS and PITELKA (1962), as well as SORENSON and BURKETT (1977) also experienced bimodal reproduction frequency when studying *Isopoda* populations from California and Texas, resp.

The number of pregnant females is observable at the bottom part of Figure 2. It can be seen that the two reproduction periods can be separated from each other quite sharply. On the upper curve this is followed with about 1–1 month shifts by the maximums of the population's individual number ($\bar{N}$), which is in relationship with the appearance of the juvenile indviduals.

Studying the body measurements of the females it can also be determined that the 2–3 years old individuals take part in the first reproduction period (with body lengths of 11.7–12, 1 mm) and the 1–2 years old ones in the second period (9.8–11,6 mm).

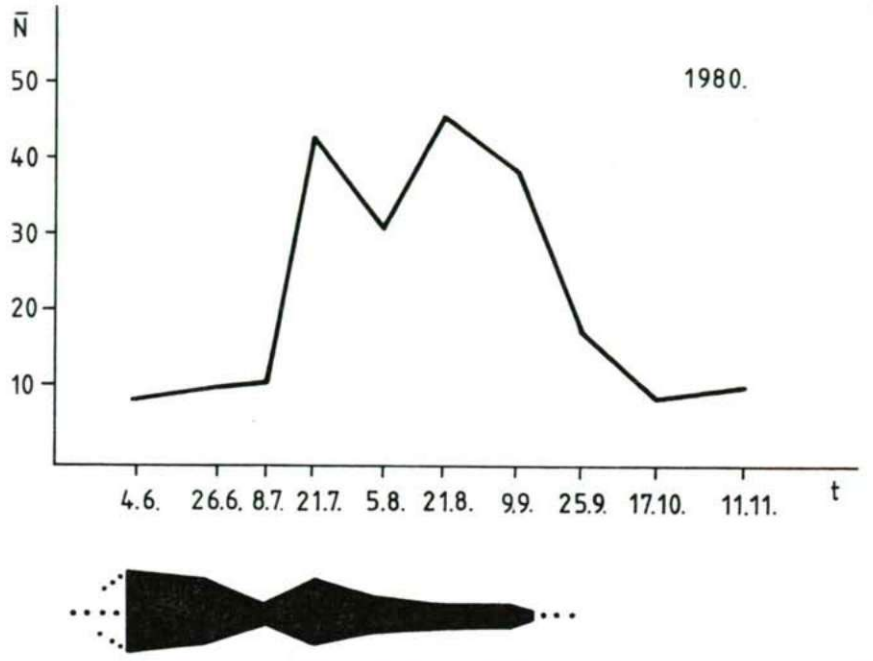


Fig. 2. Dynamics of the population and quota of pregnant females (below) on the basis of the Barber traps placed in the soil.

There are individuals — mainly among the 2-year-old ones — which become pregnant even twice a year. This was also observed by the above mentioned American authors, but in his Japanese studies, SAITO (1965) did not experience such a phenomenon in the case of the Ligidium japonicum species.

Comparing the experienced facts with the referred studies from abroad many similarities can be found in regard to the dynamics of the populations as well as in their reproduction characteristics — despite the varying climatic relations and differing species.

References

BODROGKÖZY, GY. and GYÖNGYI FARKAS (1981): Correlations between vegetation and hydroecology in the sandy grasslands of Kiskunság National Park. — Acta Biol. Szeged. *27*, 33–53.

HORNUNG, E. (1979): Makrodekomponáló ízeltlábú populációk ökológiai vizsgálata alföldi gyeptársulásokban (Ecological investigations of macrodecomposer Arthropods of grasslands in the Great Hungarian Plain). — Szeged (Thesis)

HORNUNG, E. (1981a): Investigations on the productivity of the macrodecomposer Isopod *Trachelipus nodulosus* C. L. KOCH. — Acta Biol. Szeged *27*, 203–208.

HORNUNG, E. (1981b): Data on the oxigen consumption of *Isopoda* and *Diplopoda* species. — Acta Biol. Szeged. *27*, 209–213.

KÖRMÖCZI, L., BODROGKÖZY, GY. and HORVÁTH, I. (1981): Investigation of biological production and bioclimate of sandy grasslands in Bugac (Great Hungarian Plain between Danube and Tisza). — Acta Biol. Szeged. *27*, 55–69.

MÓCZÁR, L., GALLÉ, L., GYÖRFFY, GY. and HORNUNG, E. (1980): Complex ecological investigations in a sandy soil grassland: aims and general methodology. — Acta Biol. Szeged, *26*, 161–164.
PARIS, O. H. and PITELKA, F. A. (1962): Population characteristics of the terrestrial isopod *Armadillidium vulgare* in California grassland. — Ecology, *43*, 229–248.
SAITO, S. (1965): Structure and energetics of the populations of *Ligidium japonicum (Isopoda)* in a warm temperate forest ecosystem. — Jap. J. of Ecol. *15*, 47–55.
SORENSEN, E. M. B. and BURKETT, RAY D. (1977): A population study of the Isopod *Armadillidium vulgare*, in northeastern Texas. — The Southwestern Naturalist, *22*, 375–388.
WHITE, J. J. (1968): Bioenergetics of the woodlouse *Tracheoniscus rathkei* BRANDT in relation to litter decomposition in a deciduous forest. — Ecology, *49*, 694–704.

Address of the author:
DR. ERZSÉBET HORNUNG
Department of Zoology, A. J. University
H-6701 Szeged, Egyetem u. 2.
P.O. Box 659. Hungary

Acta Biol. Szeged. 30, pp. 159–174 (1984)

SYNBIOLOGICAL STUDY OF THE LIZARD POPULATIONS OF SANDY GRASSLANDS

F. GYOVAI

Attila József University, Department of Zoology, Szeged

(Received July 20, 1983)

Abstract

In the course of a two years' study, author processed data of 634 individuals of the 3 lizard species (*Lacerta taurica, L. agilis* and *L. viridis)* living on a sandy grassland. The density of the lizard population was 557 individuals per hectare. The migration of the juvenile *L. taurica* tended towards the surrounding grazed areas. The *L. taurica* population belongs to mortality type No. II. The main mortality factor is predation. The covering by vegetation segragetes *L. taurica* from the other two populations. The consequences regarding habitude of the morphological differences between the 3 species presumably act in the direction of a decrease in interspecific competition between the individuals more than 1-year-old. The lizards represent a biomass of 1725 g/ha at the sampling area. Their annual nutriment consumption is 1.3 g/m², 18% of the complete secondary consumption, and 8% of the primary consumers' production. The lizards play a significant role in the regulation of the *Coleoptera, Aranei* and *Orthoptera* populations.

Key-words: Lacertilia, density, migration, niche analysis, interspecific competition, aliment consumption, aliment composition

Introduction

Our Department has been carrying out complex ecological investigations on sandy grasslands in the model area of Bugacpuszta since 1976 (MÓCZÁR et al., 1980). In the course of these studies, an answer was sought to questions of production-biology and ecological regulation. In this biocenosis secondary consumer populations have a significant role from the viewpoint of material- and energy-circulation, as wall as regulation (GALLÉ, 1978, FARKAS, 1978, GYENES, 1982). The present study aimed at investigating certain ecological parameters of the *Lacerta* species, reaching significant density in that area, first of all L. taurica. The author's objective was dual, viz. (i) studies on the causal background of the space-time pattern in rescept of the lizard populations; (ii) approach to the regulatory role of the biocenoses. In the present paper my studies on the population structure, the interactions between lizard populations, the biomass, the aliment composition and the alimentary consumption are summarized.

Material and methods

The studies were performed in 1981 and 1982 from March till October in a 2.38 hectare model area located at Bugacpuszta in the Kiskunság National Park. The region is an inhomogeneous sandy grassland interspersed with small sand-hill ridges and wind-furrows, and bordered by a forest at the North-eastern side. Till 1976, the area was mildly utilized for grazing, then it was isolated from

the neighbouring area, where grazing was continued. Consequently, the *Potentillo-Festucetum pseudovinae* association covering a great part of the area is turning into *Festucetum-vaginatae* associations at the sand-hill ridges, and into *Molinio-Salicetum rosmarinifoliae* associations at the wind-furrows, in the course of secondary succession. The detailed botanic, pedologic, and microclimatic characterization of the sandy grassland has mainly been comprised in the works of KÖRMÖCZI (1982a, 1982b, KÖRMÖCZI et al., 1981). I worked with manual catching in the area. Numbered aluminium boards were placed every 10 m, to mark the exact location coordinates of the catchings. Three lizard species live in the sandy grassland, namely, the *Lacerta taurica taurica* (PALLAS), the *Lacerta agilis agilis* (LINNAEUS), and the *Lacerta viridis viridis* (LAURENTI). Animals were collected at about two-week intervals. The following data were recorded: the date and place (with meter accuracy) of catching, the species, the age and the sex of the individuals as determined according to DEITY 1978. The body circumference and two body-lenght parameters viz. from the nasal tip to the cloaca orifice and from the nasal tip to the tail end, were recorded, each with mm accuracy. The second longitudinal parameter was omitted of the tail was broken or regenerated. The body weights were determined with the help of PESOLA type spring scales, with 0.1 g accuracy. The average vegetation covering the soil was estimated and the air temperature and the relative humidity (5 cm from the soil surface) were measured by a THERM 2246 type electric aspiration psychrometre.

The lizards were marked by cutting off toe phalanges in various combinations (WOODBURY 1956, BLANCHARD and FINSTER, 1933, — a modified method). The density of the lizard populations was estimated by recapturing of labelled animals according to the method of Bailey's so-called triple-cath, based on Lincoln's index (SOUTHWOOD, 1978). Samples were taken from a few stomach contents however, the catheter method (OPATRNY 1980, SMITH and MILSTEAD, 1971. LEGLER and SULLIVAN, 1979) often failed, therefore, the material of the aliment analysis was mostly formed from the stomach content of the *L. taurica* individuals which perished in the glycol insect traps on the spot.

On the 47 days spent in the field I processed, the data for 484 *L. taurica*, 169 *L. agilis*, and 228 *L. viridis* specimens comprising the data for the recaptured individuals. Eleven *L. taurica* individuals different in age and sex were collected for laboratory studies concerning aliment consumption from the grass-plots in the neighbourhood of the area. The animals were kept in 30×40 cm sized terraira and were fed with *Galleria mellonella* larvae.

Results and Discussion

1. DEMOGRAPHIC PARAMETERS

1.1. Density

The density of *L. taurica* was found rather high: in yearly average 837 ± 37 for the whole area (2,4 ha), i. e. 351 individuals per ha. The density was lower for *L. agilis* viz. $348 \pm 90/2.4$ ha, i. e. 146/ha, and still lower for *L. viridis*, viz. $144 \pm 45/2.4$, ha, i. e. 60/ha. The total density was 557 lizards per ha. This represents a biomass of 1725 g/ha, which significantly surpasses the density 700 g/ha reported by TINKLE 1972. On the contrary. the total density determined in the present study on the other hand, was by one order of magnitude lower than the average density reported by SCHOENER (0.97 individuals per m^2), which is probably the highest density measured for lizards so far (SCHOENER, 1980). It should be taken into account that Schoener studied *Anolis* lizards, which have smaller bodies, in a field where the maintaining capacity of the environment was considerably great.

1.2. Territorial behaviour and migration

The spatial structure of the lizard populations is fundamentally determined by their territorial behaviour. On the basis of the distances between the catching and recatching points, information could be obtained regarding the territory fidelity and the degree of migration of individual lizards. As shown in Table 1, the juvenile *L. tau-*

Table 1. Migration of the 3 lizard species

	L. taurica		*L. agilis*		*L. viridis*	
	juv.	ad.	juv.	ad.	juv.	ad.
$\bar{x}$ (meter) — Average of migration distance —	17.26	11.20	8.78	12.50	34.18	13.37
S (dispersion)	19.65	7.69	5.46	10.32	38.49	11.74
S^2 (variancy)	386.24	59.17	29.87	106.70	1481.00	137.98
$S/\bar{x}$	1.14	0.68	0.62	0.82	1.12	0.87
$S^2/\bar{x}$	22.36	5.28	3.40	8.53	43.34	10.31
n (number of individuals)	45	15	14	6	38	8
0–10 m (%)	64.44	46.66	64.28	50.00	52.63	37.50
11–20 m (%)	11.11	40.00	35.71	16.67	7.89	50.00
21 m< (%)	24.44	13.33	0	33.33	39.47	12.50
maximal distance	75	26	20	26	115	36

rica and *L. viridis* individuals are significantly different from the adults of the same species in respect of migration. For example, the juvenile *L. taurica* individuals have a higher average recapturing distance than the developed individuals, and their variance is also essentially higher. This latter is caused by the fact that the great majority of the individuals turned up again either within 10 m (64%), or more than 21 m (24%) apart. On the basis of the high ratio of the 0–10 m capturings, it can be assumed that the residence district of the juvenile individuals is small and that recapturings suprassing 21 m — the majority of which is between 40 and 70 m — indicate migrat-

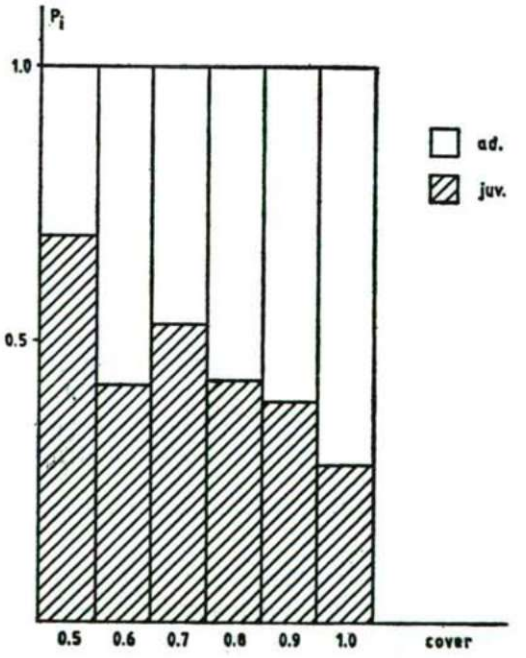


Fig. 1. Distribution of adult and juvenile individuals of *L. taurica* in the function of vegetation covering. P_i: relative frequency

ion. The adult *L. taurica* individuals appear in the 11–20 m recapturing category much more frequently (40%). The territories of these are presumably larger and less variable in size, and the migration is minimal. Presumably, the migration of the juvenile individuals supplanted from the network of the territories tended towards the surrouding grass-plots utilized for grazing where the density of the lizards is considerably lower (colonization for the occupation of "empty niche"-s (SCHOENER, 1968)). This means that juvenile lizards tending to avoid intra- and interspecific competitive effects become supplanted to areas with sparse vegetation (Fig. 1). Strong negative correlation was demonstrated between the ratio of the juvenile individuals and the covering vegetation:

$$y = 0.951 - 0.691x$$
$$r = -0.829 \qquad P < 0{,}001$$

(y = ratio of juvenile individuals, x-relative vegetation covering).
(r: correlation coefficient, P: significance).

The cause of the negative correlation may be attributed to that in the areas with higher covering, i. e. being more favourable for all three species, the juvenile *L. taurica* individuals do not tolerate the competition with *L. agilis* and *L. viridis*, and presumably predation in the case of L. viridis (WEBER, 1957, HOLEC and KMINIAK, 1970). Thus our model area, which is spared from grazing and treading functions as a pool for the *L. taurica* individuals and significantly promotes recolonization in the surrounding grass areas. All these support the view of our ecological team. This team had proposed to withdraw from grazing an area of 1–4 ha at the Bugac plain for protection of nature.

The data regarding the recapturing distances are in good agreement with the results of *Cruce* (1970). According to the present investigations, 64% of the juvenile L. taurica individuals were found within the area of 10 m diameter determined for this species on the basis of *Cruce*'s studies. For adults, *Cruce* found areas 15 m in diameter adequate. According to the present studies, 73% of the adult L. taurica individuals were refound within 15 m, and the farthest recapturing was only 26 m. These data speak against an exclusive protection of territories; they may overlap each other in this respect (RUBY, 1978). If the territory utilization were exclusive, there would be a need for about a 3-times greater area for an even space distribution of the more than 800 lizard individuals estimated in the model area. The migration of the juvenile *L. viridis* individuals showed even a higher frequency (39%). Their migration, however, did not tend towards the grazing pasture, but rather towards the neighbouring forest.

No significant age-dependence could be found for *L. agilis* individuals as to inclination to emigrate. Emigration of this species could not be demonstrated.

1.3. Age and weight structure, predational effect of the L. taurica population

The body weight and body length data for *L. taurica* individuals caught in 1982 were extrapolated to an arbitrarily chosen day (September 15, 1982), with the help of a growth curve drawn on the basis of experiences (Fig. 7). This method enabled us to study the *L. taurica* population at an exact point of time. The same could not be applied to the other two species, because the separation of the 1-year-old and older

individuals encountered difficulties and the amount of samples was lower. Of the 324 *L. taurica* individuals studied by us: 185 were juvenile. 78 one year old, 45 two years old, and 16 three years old or older (Fig. 2). The equation of the continual straight line was as follows:

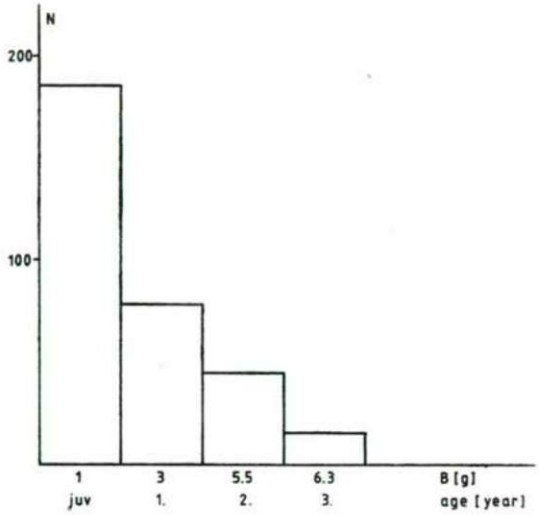


Fig. 2. Age- and weight-structure of *L. taurica* population. B(g): individual biomass, mean values expressed in grams; N: number of individuals

$$N = 216 - 54\ t \qquad r = -0.94 \qquad P \sim 0.05,$$

where N = individual number, t = age (in years).

In logarithmic form: $\ln N = 6.01 - 0.78\ t$

$$r = -0.99 \qquad P < 0.02.$$

An average body mass value could be attached to each age group, therefore, the weight structure also analysed (Fig. 2). On the basis of the weight structure:

$$N = 242.88 - 34.65\ B$$
$$r = -0.95 \qquad P < 0.05$$
$$\ln N = 5.65 - 0.40\ B$$
$$r = -0.95 \qquad P < 0.05$$

(B = boby weight expressed in grams).

According to the mortality functions on the age and weight structure, it was justified to classify the studied *L. taurica* population into mortality type No. II, which means that the logarithm of the individual numbers shows linear decrease in the function of time. Presuming that predation plays a decisive part in the mortality of this population, I performed the following study. The frequency of broken tails

is an indicator of predational pressure (TINKLE, 1972), therefore, the distribution within population of the total individuals belonging to the various weight groups (intact + regenerated + broken-tailed), and the lizards having intact tails were used in the χ^2 test (Table 2).

The assumption was therefore proved: close correlation was found between the rate of disappearance for the caught individuals, and the disappearance of lizards with intact tails. The potential predators of the lizards living in the model area are firstly birds *(Buteo, Falco, Corvus)*, perhaps mammals *(Insectivora, Mustelidae)*, and *L. viridis*.

Table 2. Ratio of the total to intact-tailed *L. taurica* individuals

Weight group (*g*)	p_i (total)	p_i (intact)
0.4–1.3	0.311	0.349
1.4–2.3	0.295	0.322
2.4–3.3	0.147	0.140
3.4–4.3	0.107	0.094
4.4–5.3	0.069	0.059
5.4–6.3	0.043	0.025
6.4–7.3	0.021	0.007
7.4–	0.002	0
	0.995	0.996

$\chi^2 = 0.0298$
$P > 0.99$

2. INTERACTIONS BETWEEN LIZARD POPULATIONS

The methods of niche analysis were used for the detailed studies on the environmental demands of the three lizard species. The justifiability of this is supported by PIANKA (1975), who pointed to the role of competition and niche relations in the organization of lizard communities. According to PIANKA and HUEY (1978), the microhabit and alimentary conditions may play a role in the niche segregation of lizards. This prompted me to study the factors determining the microhabit selection first of all. The analysis of the alimentary composition has already been performed for L. taurica.

2.1. Vegetation covering

It can be clearly seen in 3 that the vegetation covering shows irrelevant effect on *L. taurica*, while the other two species favour the regions of mass vegetation, mostly the wind-furrows. In accordance with this, the values of the niche overlapping regarding *L. taurica* are the lowest in the dimension of vegetation covering (Table 3, on the basis of the RENKONEN–SCHOENER formula). Also in conformity Fig. 3, the niche width values are the highest for *L. taurica* (Table 4). The niche centre of L. taurica is around 70% covering, in contrast to the 100% computed for the other two species. It is not the species composition of the vegetation which determines the choosing of living place, but rather the covering and structure.

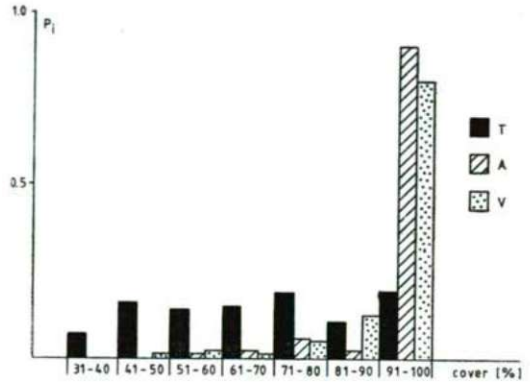


Fig. 3. Distribution of the 3 lizard species according to the vegetation covering. (T: *L. taurica*, A: *L. agilis*, V: *L. viridis*) P_i: relative frequency

Table 3. Niche overlapping of the 3 lizard species in the dimension of vegetation covering

	L. taurica	*L. agilis*	*L. viridis*
L. taurica	1		
L. agilis	0.278	1	
L. viridis	0.374	0.842	1

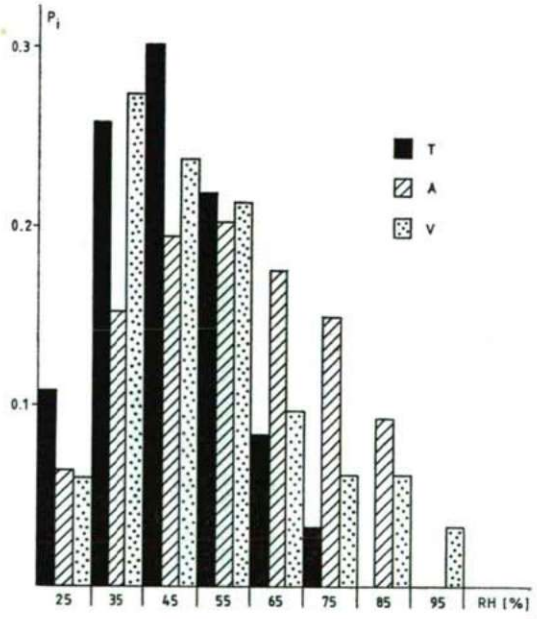


Fig. 4. Distribution of the 3 lizard species on the basis of relative humidity

2.2. Relative humidity

The histogram of this factor is illustrated in Fig. 4. The overlapping values between the three species are high in this dimension (Table 5). The niche centre for *L. taurica* is characterized by 47% relative humidity: for *L. agilis* and *L. viridis* the

Table 5. Niche overlapping of the 3 lizard species on the basis of RH %

	L. taurica	*L. agilis*	*L. viridis*
L. taurica	1		
L. agilis	0.694	1	
L. viridis	0.818	0.762	1

corresponding percentages are 56% and 51% respectively. There is no significant variation in niche width between the three species. Table 4 comprises the values of the niche width calculated by SHANNON's formula expressed in ln, regarding the three lizard species, in the studied three dimensions.

2.3. Temperature

The studies regarding air temperature do not show segregation of the three populations (Fig. 5, Table 4). (Here a role can be attributed to the colour and structure of the sand soil surface, due to the varying degree of reception of isolation). The niche centres per species, viz. *L. taurica:* 27.8 °C, *L. agilis:* 26,7 °C, *L. viridis:* 26.3 °C, show rather slight variations. Comparing these data with the relevant results of the studies accomplished by KORSÓS (1982a, b), the following determinations could be made regarding *L. agilis* and *L. viridis*. The niche width of these two species is closely similar to the niche width obtained in the present study, nevertheless, in the latter

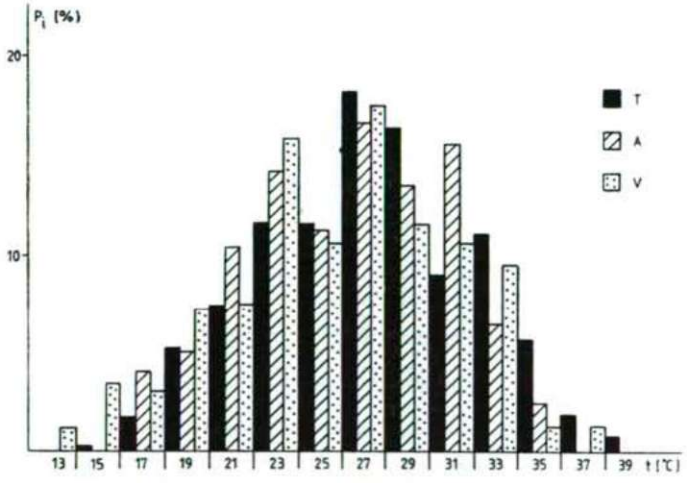


Fig. 5. Distribution of the 3 lizard species according to temperature

Table 4. Niche width values for the 3 lizard species in the three studied dimensions

	B%	RH%	t (°C)
L. taurica	3.17	2.27	2.24
L. agilis	0.44	2.49	2.16
L. viridis	0,72	2.43	2.28

case, a conspicuous shift towards higher temperatures was experienced (+10 °C for *L. agilis*, +4 °C for *L. viridis*). This might be attributed to the different character and climate, of the areas studied by us.

As shown by the studies on the three niche dimensions, the vegetation covering causes a very large segregation between the three populations. The lizards choosetheir place of living on the basis of the mass of the vegetation (SCHOENER, 1978).

2.4. Morphological differences

The morphological differences may have a potential role in the separation of species according to habit (KORSÓS, 1982b). Table 6 demonstrates the values of the two characteristic body ratios by species; namely, the tail length per body length

Table 6. Body ratio values for the 3 lizard species

	L. taurica	*L. agilis*	*L. viridis*
F/L S (dispersion)	1.651 0.103	1.668 0.117	1.915 0.089
K/L S (dispersion)	0.503 0.048	0.579 0.039	0.566 0.025
Morphological characterization	short-tailed slim	short-tailed squat	long-tailed squat

(F/L) and body width per body length (K/L) ratios. In these studies each samples consisted of 30 individuals irrespective of age, fordoby ratios of did not distinguish juvenile lizards from developed ones. PLETICHA (1968) and KORSÓS (1981) found isometric postembryonal growth. The F/L constant for L. taurica and *L. agilis* can sharply be distinguished from the at for *L. viridis*. Due to its greater dispersion, the K/L ratio shows some difference — although not an unambiguous one — between sand lizard, and the other two species.

A close correlation has been demonstrated in respect to the lizard's jaw length and the size of the prey (KORSÓS, 1982b). I compared in this context the body length with the aliment proportions from the data pairs of 110 L. taurica and its prey. The relationship found between these can be demonstrated by the following function:

$$y = -3.84 + 0.22\,x$$
$$r = 0.570 \qquad P < 0.001$$

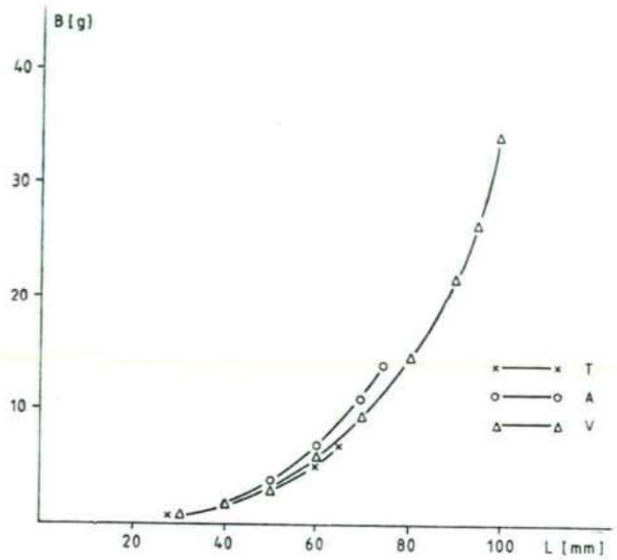


Fig. 6. Body length and weight curves for the 3 lizard species L: body length

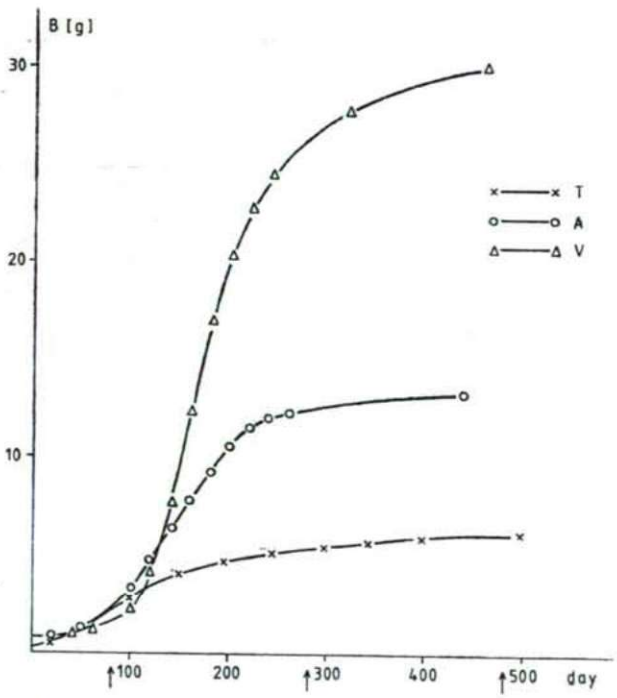


Fig. 7. Growth curves for the 3 lizard species (↑ =hibernation)

where y = dimension of prey in mm, X = the body length of *L. taurica* in mm. On the basis of the close correlation between the prey dimension and jaw length SCHOENER and GORMAN refer to relationships regarding competition (SCHOENER and GORMAN 1968). In respect of the evolution of the interspecies differences in body size DUNHAM and TINKLE 1978 also propound the role of competition.

Since the correlation shown in Fig. 6 exists between the body length and weight of the three lizard species, the measures of the prey can accordingly be examined in the function of body weight. It is striking in Fig. 6 that the curve of *L. agilis* runs above that of *L. viridis*, and the curve of *L. taurica* runs below it. Here I refer back to the K/L values and the morphological characteristics demonstrated in Table 6. Accordingly competition regarding aliment only exists between individuals of similar body weight. In this respect, therefore, interspecific competitive interaction can only be determined between juvenile individuals in autumn and spring. The reason for this is the different growth rate of the three species (Fig. 7). Following the spring of the first hibernation, the relative growth of all three species shows a strong outset (CRUCE, 1970, regarding *L. taurica*) (Fig. 8). Nevertheless, in respect of body weight and body length *L. agilis* strongly, and *L. viridis* rather intensely surpass *L. taurica*. It has been shown that in regard to the obtainment of food the 1-year-old or older lizards of varying species do not exclude each other competitively. If later on, certain degree of competition arises between them — interactions of growth curves — this is limited to a rather short period.

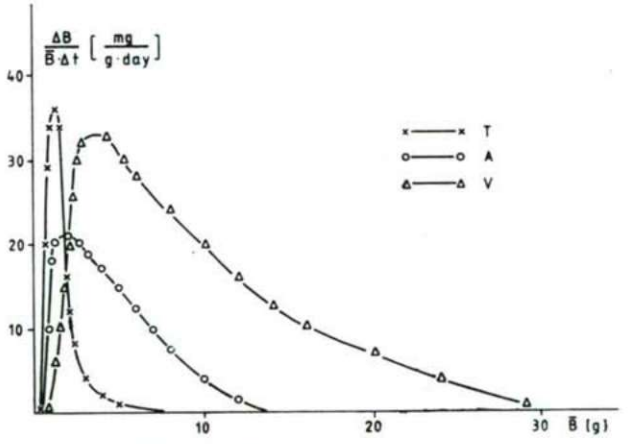


Fig. 8. Relative growth of the 3 lizard species
$\frac{\Delta B}{\bar{B} \cdot \Delta t}$: relative increase in body weight

3. THE PLACE OF LIZARDS IN THE ALIMENTARY NETWORK

3.1. Individual biomass changes and food consumption

Here the aim was not a detailed analysis of production-biology, but only the demonstration of the laboratory studies related to the food consumption of the characteristic lizard species of the model area, viz. *L. taurica*. Fig. 9 shows a correlation

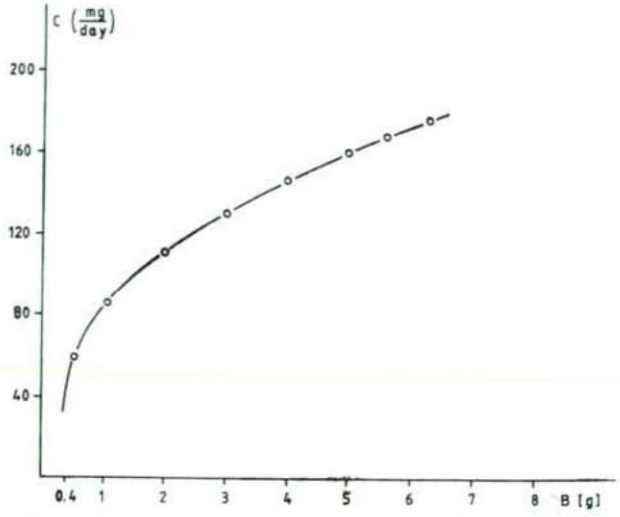


Fig. 9. Individual biomass changes and food consumption of *L. taurica*
C: aliment consumption (fresh weight mg per day)

between the changes in biomass and food consumption. With the x–y value pairs of this graph the following logarithmic function was obtained:

$$\ln C = 4.32 + 0.479 \ln B$$
$$r = 0.984 \qquad P < 0.001$$
$$C = 75.24\ B^{0.479}$$

B = live weight (gram)
C = food consumption (fresh weight mg per day)
C' = food consumption (dry weight mg per day) = 0.205 C

The function is in good agreement with the relevant results of the work of AVERY (1978), who author reported on the following relationship: at low temperature: $C' = 19.3\ B^{0.71}$, at high temperature: $C' = 34{,}6\ B^{0.65}$. Despite the similar course of our functions, the experienced variations were presumably caused by differences in the animals of alimentation as well as the studied objects (*Podarcis muralis* and *P. sicula*), since the average active body temperature of these species was 33.6 °C and 35.2 °C, respectively, i. e. about 8 °C higher than that in the case of the *L. taurica* regarding the present study. Thus, the food consumption of the *Podarcis* species is almost the double that of *L. taurica*.

In the knowledge of the weight structure, density and consumption of *L. taurica* population ,the biomass and average daily food consumption values of this species can be calculated for the model area. For the other two species the biomass and the aliment consumption values were estimated by extrapolation. The annual average biomass values were as follows:

$$B_{(L.\ taurica)} = 831 \text{ g/ha}$$
$$B_{(L.\ agilis)} = 584 \text{ g/ha}$$
$$B_{(L.\ viridis)} = 360 \text{ g/ha}$$
$$B_{(total)} = 1725 \text{ g/ha.}$$

In comparison with other studies, this is a rather high total biomass value. TINKLE (1972), e. g. reported 700 g/ha. Values of the daily food consumption:

$$C_{(L.\ taurica)} = 36.1 \text{ g/ha} \cdot \text{day}$$
$$C_{(L.\ agilis)} \approx 20.4 \text{ g/ha} \cdot \text{day}$$
$$C_{(L.\ viridis)} \approx 10.2 \text{ g/ha} \cdot \text{day}$$
$$C_{(total)} \approx 66.7 \text{ g/ha} \cdot \text{day}.$$

The period of activity of the lizards can be estimated to 200 days per year, thus their annual consumption amounts to 1.334 g/m². Taking into account the consumption by the other carnivores living in the model area (firstly, *Aranei, Formicoidea*) we may state lizards take up 18% of the food amount flowing into the secondary consumption level, 8% of the production regarding the primary consumption level, and 0.66% of the primary production (FARKAS, 1978; GALLÉ, 1978, GYENES, 1982).

3.2. Qualitative composition of aliment

Table 7 summarizes the results of the analysis of the stomach content of 24 *L. taurica* individuals. Trophic levels: primary consumers (1), secondary consumers (2), decomposers (3), sustinents (4). Fig. 10 illustrates the percentual quota of the prey listed into the four trophic levels, regarding aliment.

The diversity value of the prey (SHANNON and WEAWER 1949) is the following: $H(S) = 2.28$. The highest reachable value here would be $H(S)_{max} = 2.89$.

$$\frac{H(S)}{H(S)_{max}} = 0.78$$

Table 7. Stomach content of 24 *L. taurica* individuals

Taxon	Trophic level	*n* (individual number)	%
Gastropoda	3	3	2.65
Collembola	3	7	6.19
larva (indet)	1	3	2.65
larva (indet)	2	1	0.88
larva (indet)	3	1	0.88
Blattidea	3	3	2.65
Mantidea	2	1	0.88
Orthoptera	1	11	9.73
Heteroptera	1	3	2.65
Homoptera	1	7	6.19
Neuroptera	2	2	1.77
Coleoptera	1	34	30.09
Coleoptera	2	0	0
Coleoptera	3	2	1.77
Lepidoptera	1	1	0.88
Diptera	3 and 4	3	2.65
Hymenoptera	4	1	0.88
Formica	2	9	7.96
Aranei	2	21	18.58
Total:		113	99.93

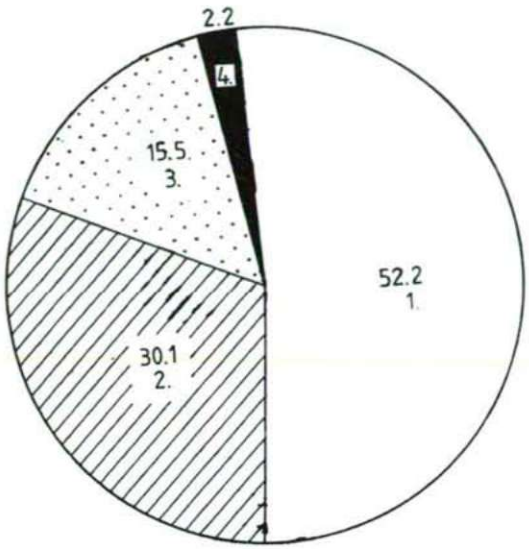


Fig. 10. Percentual composition of the aliment of *L. taurica*.
1: primary consumers, 2: secondary consumers, 3: decomposers, 4: sustinents

This means that the aliment composition of this species is variable. The diverse aliment composition and the specialization of low degree is presumably a general characteristic of *Lacertidae* (ROBINSON and CUNNINGHAM 1978). The above values were determined for relatively large taxonomic categories, lizards choose prey on the ground of the general appearance, motility and dwelling of the prey; and they do not take its species into account. Table 7 shows that insects flying well (for example *Diptera, Lepidoptera, Neuroptera,* etc.), that is, those difficult to catch represent a slight proportion of the in consumption. *L. taurica* may play a significant role in the control of phytophage beetles and spiders. According to BALLINGER (1979), in the periods abundant in nutriment lizards "change to" the consumption of aliments of larger bodies. It may be assumed, therefore that at the time of the mass increase of the Locusts, the lizards of Bugac also play a role in the regulation of these.

Acknowledgement

Author expresses his sincere thanks to DR. LÁSZLÓ GALLÉ senior researcher for his devoted help given during my studies, to the co-workers of the Ecological Laboratory of the Department of Zoology, Attila József University, for their kind help, and also to DR. LÁSZLÓ KÖRMÖCZI, for the phytological informations.

References

AVERY, R. A. (1978): Activity patterns, thermoregulation and food consumption in two sympatric lizard species (*Podarcis muralis* and *P. sicula*) from central Italy. — J. Anim. Ecol., *47 (1)*, 143–158.

BALLINGER, R. E. and BALLINGER, R. A. (1979): Food resource utilization during periods of low and high food availability in *Sceloporus jarrovi (Sauria: Iguanidae)*. — The Southwestern Naturalist *24*, 347–363.

BLANCHARD, F. N. and FINSTER, E. B. (1933): A method of markong snakes for future recognition with a discussion of some problems and results. — Ecology, *14*, 334–347.

CRUCE, M. (1970): The seasonal and diurnal activity cycle in the lizard Lacerta taurica. — Studii Cerc. Biol. Acad. R. P. R. Ser. Zool. *22*, 467–472. (rom.)

DELY, O. GY. (1978): Hüllők — *Reptilia*. Fauna Hung. 130.

DUNHAM, A. E. and TINKLE, D. W. (1978): Body size in island lizards: a cautionary tale. — Ecology, *59*, 1230–1238.

FARKAS, L. (1978): Araneae vizsgálatok gyepökoszisztémákban. (Araneae studies in grass-ecosystems.) — Thesis, 1–32.

GALLÉ, L. (1978): Formicoidea populációk denzitása és produktivitása gyepökoszisztémákban. (Density and productivity of Formicoidea populations in grass-ecosystems.) — Dissertation for Candidate Degree, 1–125.

GYENES, K. (1982): Tettigonoidea populációk ökológiai paramétereinek vizsgálata homokpusztai gyepen. (Studies on the ecological parameters of Tettigonoides populations in sandy grasslands.) Thesis, 1–45.

HOLEC, P. and KMINIAK, M. (1970): Zur ökologie der Art *Lacerta viridis* auf dem Gebiet der Devinska Kobyla (bei Bratislava). — Biologia, Bratisl., *25*, 805–810.

KORSÓS, Z. (1981): Szabadföldi vizsgálat két gyíkpopuláción. (Field studies on two lizard populations.) — Paper submitted to Sciencific Students Circle, Eötvös Loránd University of Sciences, 1–11.

KORSÓS, Z. (1982a): Field observations on two lizard populations (*Lacerta viridis* and *L. agilis*). — Vertebrata Hungarica, *21*, 185–194.

KORSÓS, Z. (1982b): A zöld gyík és a fürge gyík összehasonlító niche-vizsgálata. (Comparative niche-studies on green lizard and sand-lizard.) — Thesis, Eötvös Loránd University of Sciences, 1–109.

KÖRMÖCZI, L. (1982a): Összefüggések homokpusztai gyepek zonációja és talajuk fizikai-kémiai kondíciója között Bugacon. (Relationships between the zonation of sandy grasslands and the physical-chemical condition of their soil at Bugac.) — Acta Biol. Szeged.

KÖRMÖCZI, L. (1982b): A felsőbugacpusztai homoki gyeptársulások synökológiai analízise. (Synecological analysis of sandy grassland communities at Upper Bugac Puszta.) — Doctoral Dissertation, Szeged, 1–99.

KÖRMÖCZI, L., BODROGKÖZY, GY. and HORVÁTH, I. (1981): Investigation of biological production and biochlimate of sandy grasslands in Bugac (Great Hungarian Plain, Between, Danube and Tisza). — Acta Biol. Szeged. *27*, 169–179.

LEGLER, J. M. and SULLIVAN, L. J. (1979): The application of stomach-flusing to lizards and anurans. — Herpetologica, *35*, 107–110.

MÓCZÁR, L., GALLÉ, L., GYÖRFFY, GY. and HORNUNG, E. (1980): Complex ecological investigations in a sand soil grassland: aims and general methodology. — Acta Biol., Szeged. *26*, 161–164.

OPATRNY, E. (1980): Food sumpling in live Amphibians. — Vest. es Spolec, Zool. *44*, 268–271.

PIANKA, E. R. (1975): Niche relations of desert lizards. — in: Cody — Diamond: Ecology and evolution of communities, Harvard, Univ. Press, pp. 292–314.

PIANKA, E. R. and HUEY R. B. (1978): Comparative ecology, resource utilization and niche segregation among gekkonid lizards in the southern Kalahari. — Copeia, 691–701.

PLETICHA, P. (1968): Das relative Wachstum der Zauneidechse *Lacerta agilis*. — Zool. Listy. *17*, 63–74.

ROBINSON, M. D. and CUNNINGHAM, A. B. (1978): Comparative diet of two Namib Desert sand lizards *(Lacertidae)*. — Madoqua, *11*, 41–53.

RUBY, D. E. (1978): Seasonal changes in the territorial behavior of the Iguanid lizard *Sceloporus jarrovi*. — Copeia, 430–438.

SCHOENER, T. W. (1968): The *Anolis* lizards of Bimini: resource partitioning in a complex fauna. — Ecology *49*, 704–726.

SCHOENER, T. W. and GORMAN, G. (1968): Some niche differences in three Lesser Antillean lizards of the genus *Anolis*. — Ecology *49*, 819–830.
SCHOENER, T. W. and SCHOENER, A. (1980): Densities, sex ratios and population structure in four species of Bahamian *Anolis* lizards. — J. Anim. Ecol. *49*, 19–53.
SHANNON, C. D. and WEAWER, W. (1949): The mathematical theory of communication, Baltimore.
SMITH, D. D. and MISTEAD, W. (1971): Stomach analysis of the orevice spiny lizard *(Sceloporus poinsetti)*. — Herpetologica *27*, 147–149.
SOUTHWOOD, T. R. E. (1978): Ecological methods. — Chapman and Hall, London.
TINKLE, D. W. (1972): The dinamics of a Utah populations of *Sceloporus undulatus*. Herpetologica *28*, 351–359.
WEBER, H. (1957): Vergleichende Untersuchungen des Verhaltens von *Lacerta viridis, L. muralis* und *L. lepida*, — Z. Tierpsychol. *14*, 448–472.
WOODBURY, A. M. (1956): Marking amphibians and reptiles. – Ecology *37*, 670–675.

Address of the author:
F. GYOVAI
Department of Zoology, A. J. University
H-6701 Szeged, P.O. Box 659.
Hungary

Acta Biol. Szeged. 30, pp. 175–190 (1984)

COMPARISON OF SERIES FROM THE AVAR AGE BY CLUSTERING

GY. FARKAS AND P. HUNYA

Attila József University, Department of Anthropology,
Kalmár Laboratory of Cybernetics, Szeged

(Received June 30, 1983)

Abstract

Author determined the weighted mean and standard deviation of 14 measurements from 28 Avar-Period series dug up in Hungary. Their comparison with the HOWELLS mean-sigmas manifested significant differences in 13 male and 8 female characters.

With the help of computer the PENROSE distance were determined (of size and shape; generalized) and by simple (single-link) and complex (complete-link) clustering, the distances between the various series were demonstrated.

Key-words: Avar-Period series, mean sigma. PENROSE distances, clustering

Introduction

The evaluation of human bone remains from the many Avar-Period cemeteries excavated on Hungarian territory was firstly carried out from the viewpoint of taxonomy and facial-flatness. Synthesizing studies provide survey on the sites (ÉRY, 1968) and on the results of the biometrical comparison of the 6th–12th century cemeteries (ÉRY, 1970). The latter publication comprises the studies on 10 measurements and 7 indices of 34 male and female series, taking into account the mean sigmas of VAN BORK-FELTKAMP, HOWELLS and ALEKSEEV–DEBEC. The 909 male and 779 female skeletons of the 16 Avar-Period series, and the 18 series belonging to the time of the Hungarian conquest and the subsequent Arpadian Age practically form a combined europid sample (with the exception of the Üllő I and II sites). However, they provide less information on the possible differences between Avar Age series.

With the purpose to determine this, similar studies were accomplished on 28 Avar-Period series, mainly originating from the area between the rivers Danube and Tisza.

Materials and methods

The findings from the following cemeteries were taken into consideration:

Adorján-Highway (Adorján-A) and Adorján-farm (Adorján-F) (BARTUCZ–FARKAS, 1957), Alattyán-Tulát (WENGER, 1957), Áporkai-Ürbőpuszta (LIPTÁK, 1951), Bačka-Topola (FARKAS–MARCSIK, under publication), Csákberény (TÓTH, 1962), Előszállás-Bajcsihegy (WENGER, 1967), Győr (NEMESKÉRI, 1943), Homokmégy–Halom (LIPTÁK, 1957), Jánoshida–Tótkérpuszta (WENGER, 1953), Kecel-I (LIPTÁK, 1954), Kékesd (WENGER, 1968), Kiskőrös–Pohibuj (LIPTÁK, 1956), Kun-

szállás (LIPTÁK–VARGA, 1971), Madaras (LIPTÁK–MARCSIK, 1976), Mélykút (MARCSIK, 1971), Mosonszentjános (LIPTÁK, 1959), Szolnok–Rákóczifalva (LIPTÁK–MARCSIK, 1975), Sükösd (KŐHEGYI–MARCSIK, 1971), Szeged–Fehértó–A (LIPTÁK–VÁMOS, 1969), Szeged–Kúndomb (LIPTÁK–MARCSIK, 1966), Szeged–Makkoserdő (VÁMOS, 1973), Szekszárd–Palánk (LIPTÁK, 1974), Szentes–Kaján (WENGER, 1955), Szarvas–Kákapuszta (LIPTÁK–MARCSIK, 1971), Tiszavárkony (LIPTÁK, 1958), Üllő-I, and II (LIPTÁK, 1955).

The analysis was carried out on the basis of the craniometric numeration according to MARTIN–SALLER (1956), with the following measurements: 1, 8, 9, 17, 38, 45, 47, 48, 51, 52, 54, 55, 66 and 69. The series in which any of the 14 measurements was missing or the individual data could not be substituted were not included in the joint sample.

According to our basic conception the 28 Avar-Period series were regarded as a single population. As the first step the sample element numbers (n), the arithmetic mean ($\bar{x}$) and standard deviation (s) were repeatedly determined for all 14 types from the basis data by sex. The same calculation was carried out for literary data for which above parameters had been given. Taking into account these parameters, we gained the combined sample element numbers (N), the weighted arithmetic mean ($\bar{x}$) and the wighted standard deviation (S) according to character and sex. These are given in Table 1. An R-40 type computer was used.

To determine the variation and their degree between the HOWELLS-type mean-sigmas and standard deviations obtained for the Avar-Period findings, the chi-square probe of Rao was used:

$$\varkappa^2 = \frac{n.s^2}{\varrho^2},$$

where n = the number of the appropriate characters in the combined sample; s^2 = variance of the combined sample; and ϱ^2 = the HOWELLS-type mean-sigma referring to the appropriate character.

The difference obtained on the basis of the $\sqrt{2.\varkappa^2} - \sqrt{2n-1}$ formula follows normal distribution above 30 degrees of freedom around O mean, with unit standard deviation. On this basis, if the above difference value reaches 2 and 3, the difference should be regarded as significant and highly significant respectively (THOMA, 1957).

The results of calculations are presented in Table 2.

For the calculation of the PENROSE-distances the combined sample's standard deviation was regarded as Avar-Period mean-sigma, in our opinion, the HOWELLS-type mean-sigma values published in the literature (THOMA, 1957) cannot be applied to the Avar-Period series, because the standard deviations regarded as standards were only calculated from europid-type series. Furthermore, the standard deviation values are not distinguished according to sexes.

Also by means of computer, the PENROSE-distances (KNUSSMANN, 1967) — according to size, shape, and generalized — between the samples were determined separately for males and females. On the basis of these, simple (single-link) and complex (complete-link) clusters were formed by computer. The results are given in Figures 1–12.

In the Figures the following symbols were used: MCH means the size distances for males, FCH the same for females; MCF (males) and FCF (females) for shape distances; MCR (males) and FCR (females) for the generalized distances.

With single-link clustering those two samples were regarded as belonging to one group between which a linkage could be determined whose each element reaches a given level.

The complete-link relation brings into prominence the groups in which each sample is in close connection with the others. This method regards two samples as belonging to one group if both represent a similarity level higher than a given level with every element of the group, and with each other.

Results

Table 2 gives information on the differences between the standard deviation values of the HOWELLS-type mean-sigmas and those of the Avar-Period combined sample.

In the case of males significant difference was not obtained concerning the width of the lower jaw (go-go); for the other 13 parameters the standard deviations of the Avar Age findings show greater variation than the mean-sigmas given by

HOWELLS. As regards females, significance was in case of five measurements: maximum length of the skull, minimum forehead width, morphologic face height, height and width of orbita.

We concluded that the differences of the standard deviations regarding males could first of all arise from the mongolid character and unambigously proves our assumption, that the HOWELLS-type mean-sigmas calculated for europids cannot always be applied to the Avar-Period series, which comprise mongoloid elements.

In the case of females, on the other hand, the slighter variations may arise from the fact that the HOWELLS-type values refer to males.

As to the size distances (MCH) for the male findings the similarily was the most striking between the Homokmégy and Kecel-I sites, while the findings excavated at Rákoczifalva could only be linked to the rest of the samples at the 0.60 level. On the basis of the size distances, further sites can also be divided into groups (Fig. 1.)

On the basis of the size distances (FCH) the female skeletons show the greatest similarity at the 0.95 level, in the case of the Homokmégy, Kecel–I and Üllő–II series, while the slightest link, 0.65, to the rest of the samples was found from the findings at Szarvas (Fig. 2).

With the complete-link clustering an entirely separate group is formed by the male findings of the mainly mongolid series from Bačka–Topola, Madaras, Szarvas, Kunszállás and Mosonszentjános. The series Adorján–F segregates. The group of findings from Rákóczifalva is entirely different from every other sample. The rest of the series can be ranked into a larger group. Within the first and third groups at a lower level, subgroups segregating from each other were observed (Fig. 3).

With completeste clustering in the case of the female findings, the Avar Age findings from Szeged–Makkoserdő and Szarvas can sharply be separated, and the rest of the series can be divided into two large groups, within which subgroups can be distinguished (Fig. 4).

Fig. 5 gives information on the shape distances regarding males (MCF). Further three series (Tiszavárkony, Üllő–I and II) could be linked to the group formed by the two sites (Homokmégy and Kecel–I) connectible on the basis of the size distances. In this case, too, the Avar Age findings from Rákóczifalva were those less connectible to the rest of the series. Apart from the aforesaid, further four major groups could be separated.

In the case of females (FCF) the highest (of 0.95 level) and lowest (0.65 level) linkage could be formed in entire similarity to the results obtained regarding the size distances (Fig. 6).

With the complete-link clustering the male findings (Fig. 7) could be divided into a smaller (Bačka–Topola, Mélykút, Madaras, Kunszállás, Mosonszentjános) and a larger group comprising the majority of the series. The Rákóczifalva series forms a completely separate one. The result of the clustering is greatly similar to the result of the complete-link clustering gained for the size distances, only the Adorján-F site does not form a separate group as regards shape distance.

Fig. 8 shows the sketch of the complete-link clustering of the shape distances in the female series. On this basis, the samples can be divided into two completely different groups. Within the larger group, considering levels between 0.30 and 0.75, five subgroups of findings could be separated, being in connection with each other.

Fig. 9 demonstrates the obtained information on the relationship of the male series (MCR) on the basis of the generalized distances. It could be determined that the

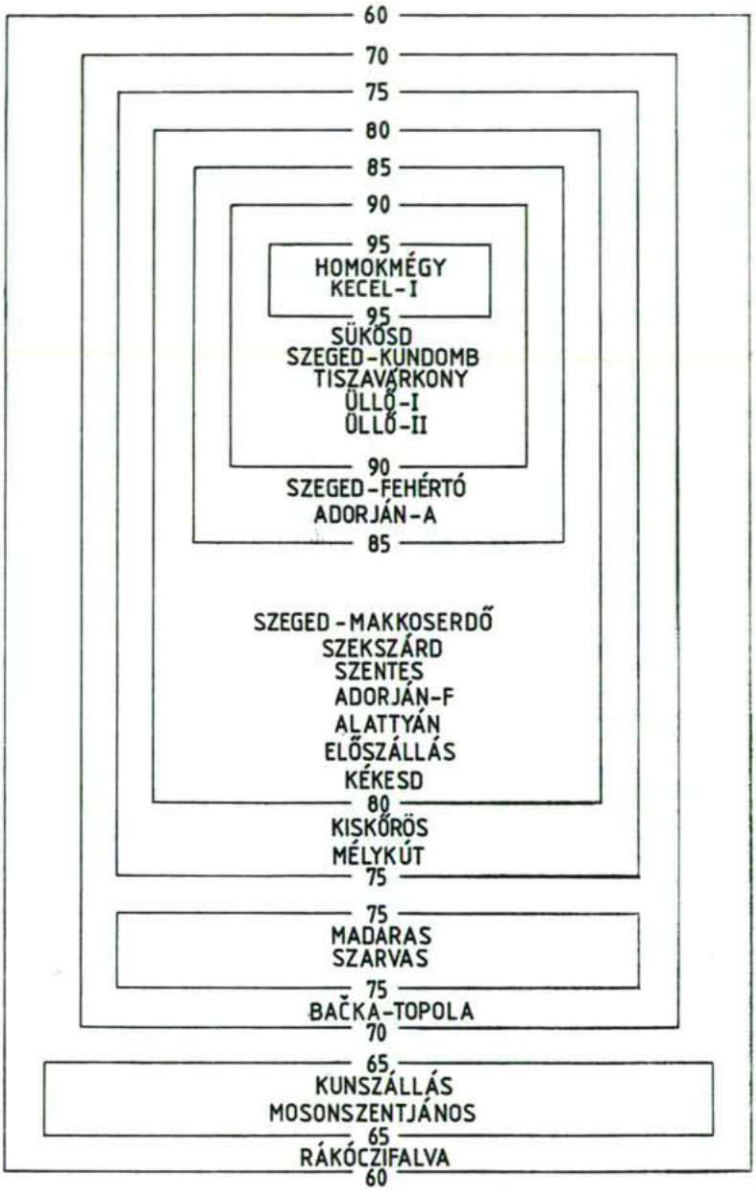


Fig. 1. Results of simple clustering on the basis of the size distances regarding male findings (MCH)

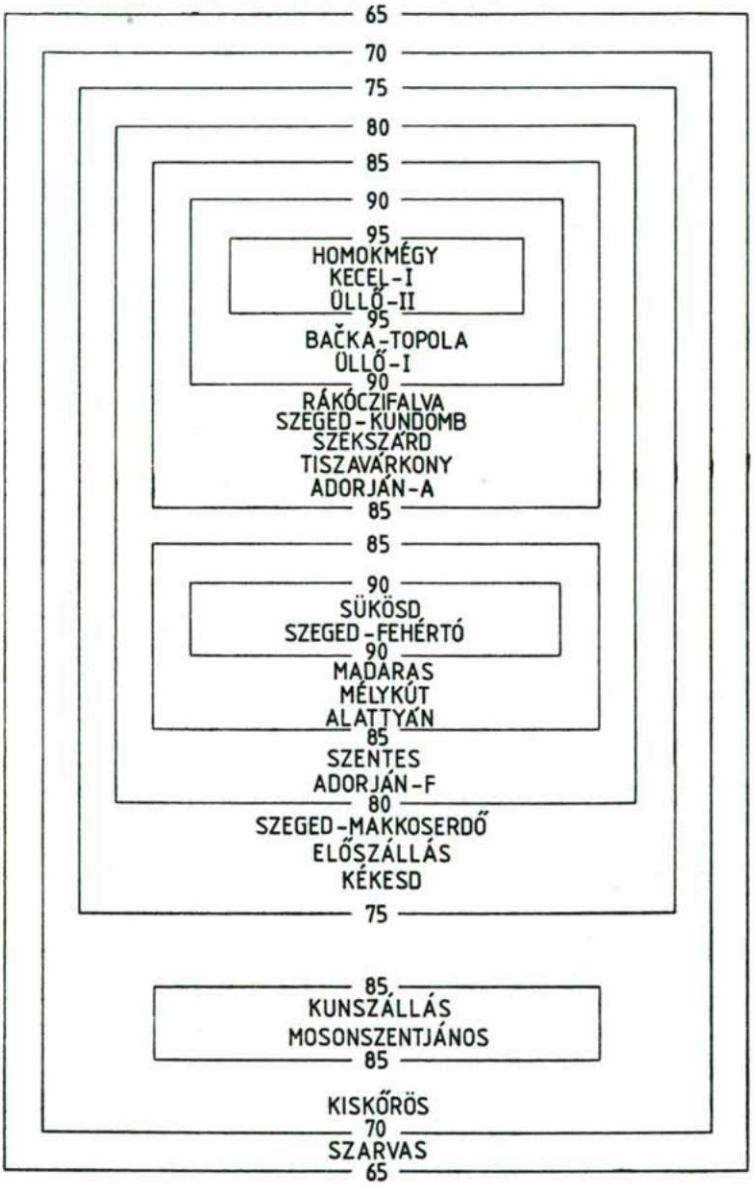


Fig. 2. Result of simple clustering on the basis of the size distances regarding female findings (FCH)

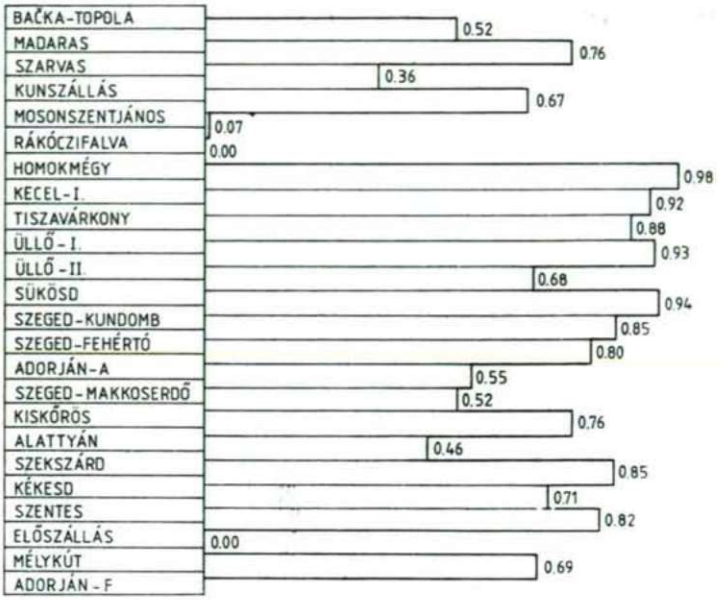


Fig. 3. Result of complex clustering on the basis of the size distances regarding male findings (MCH)

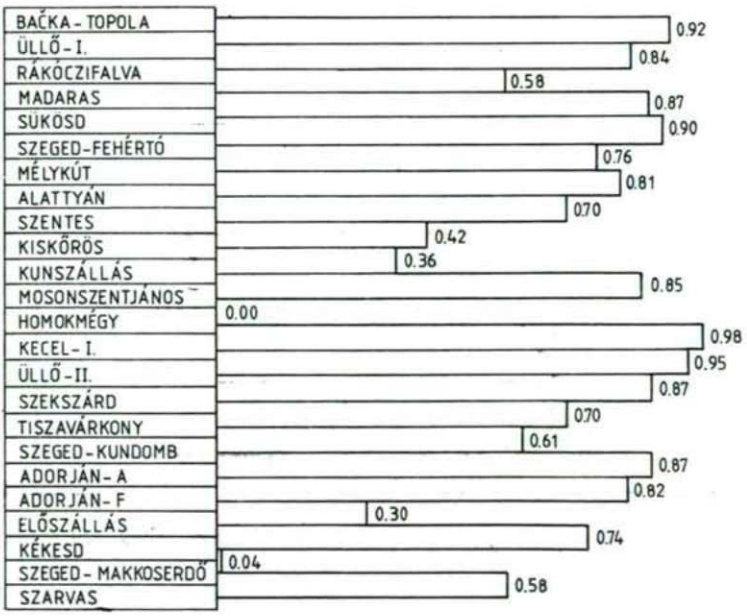


Fig. 4. Result of complex clustering on the basis of the size distances regarding female findings (FCH)

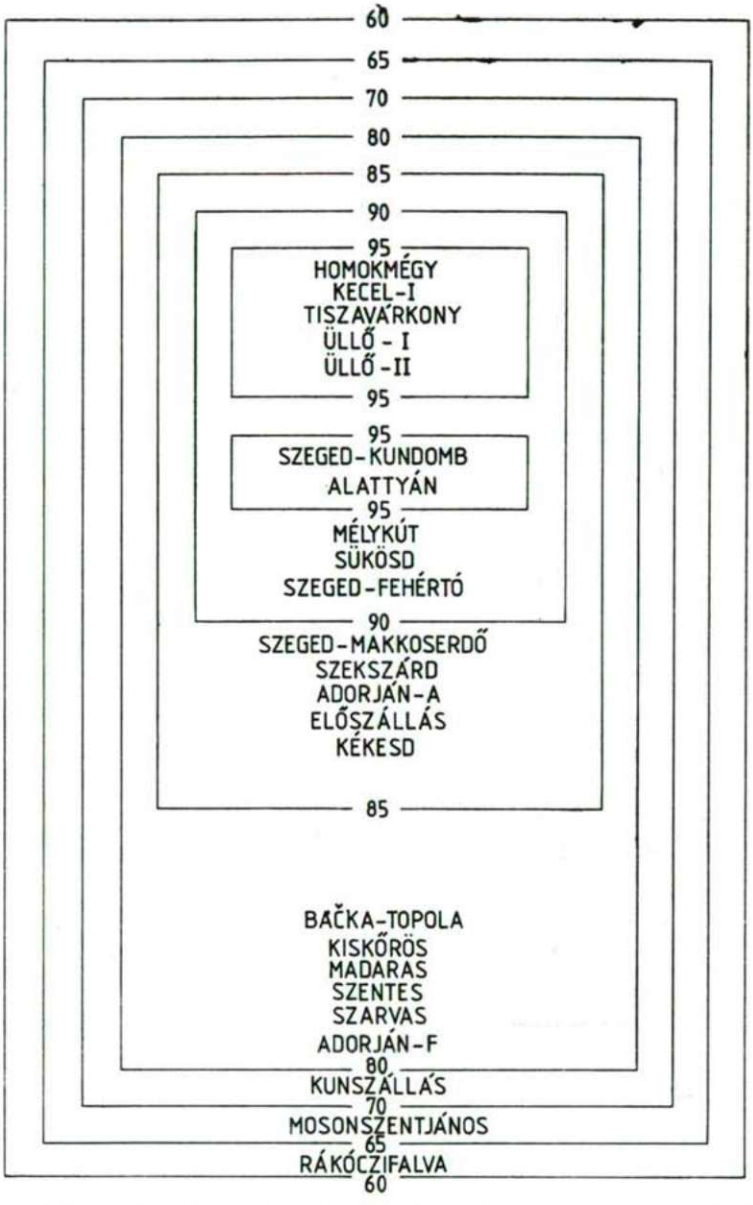


Fig. 5. Result of simple clustering on the basis of the shape distances regarding male findings (MCF)

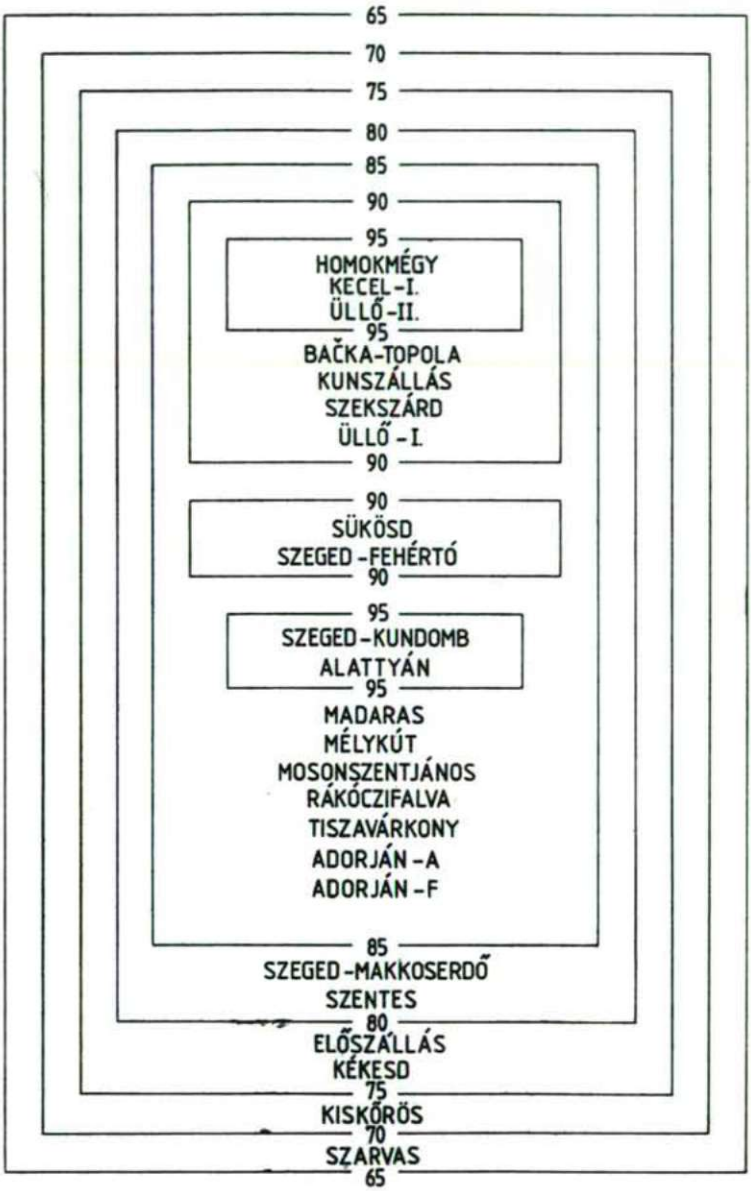


Fig. 6. Result of simple clustering on the basis of the shape distances regarding female findings (FCF)

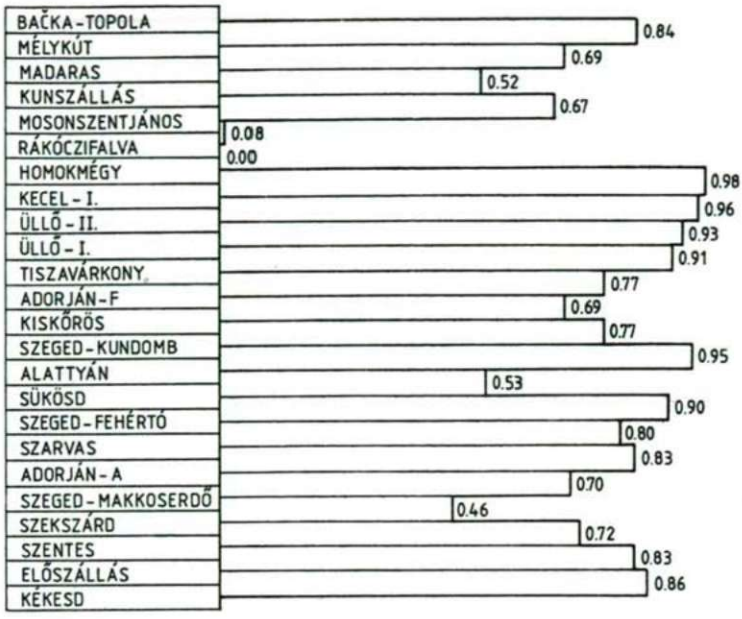


Fig. 7. Result of complex clustering on the basis of the shape distances regarding male findings (MCF)

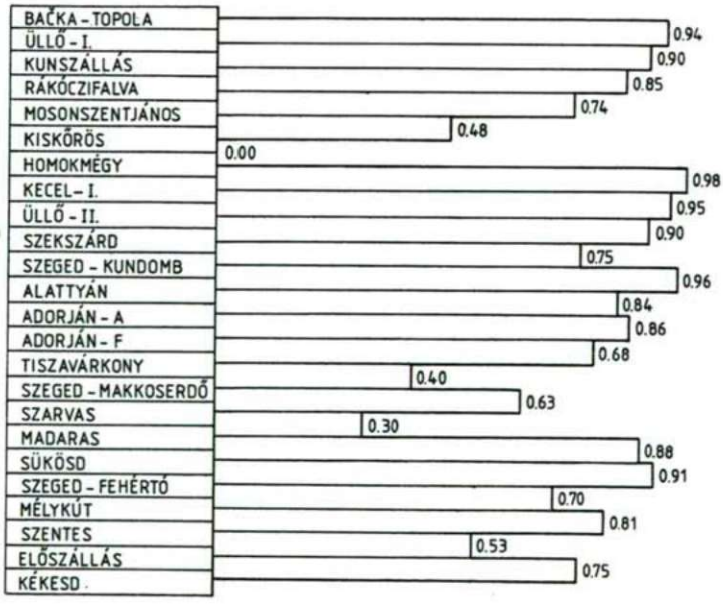


Fig. 8. Result of complex clustering on the basis of the shape distances regarding female findings (FCF)

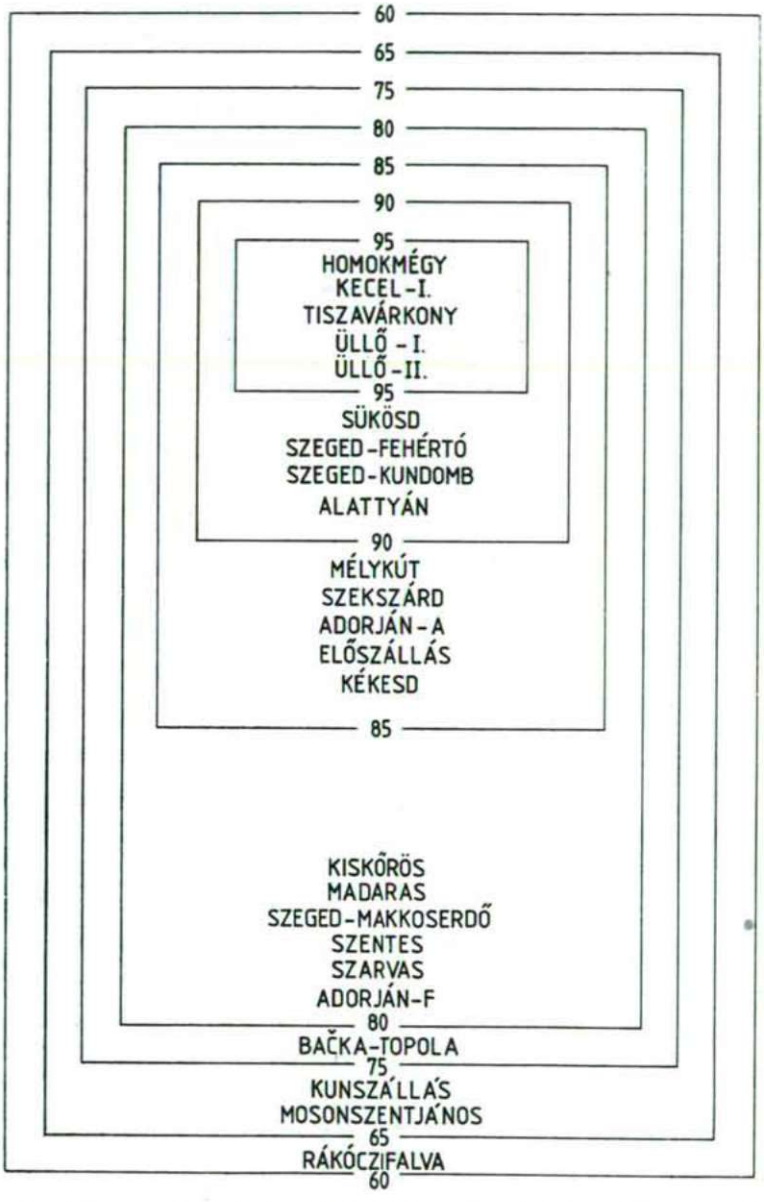


Fig. 9. Result of simple clustering on the basis of the generalized distances regarding male findings (MCR)

Fig. 10. Result of simple clustering on the basis of the generalized distances regarding female findings (FCR)

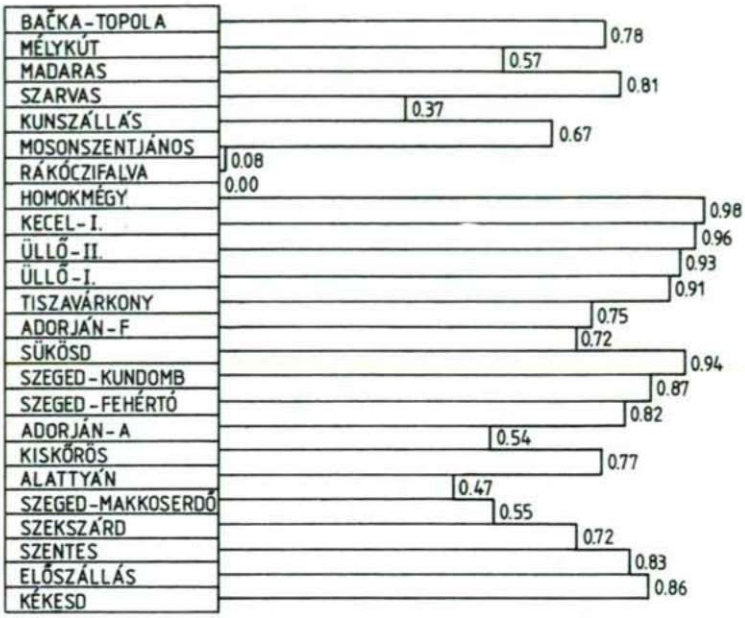


Fig. 11. Result of complex clustering on the basis of the generalized distances regarding male findings (MCR)

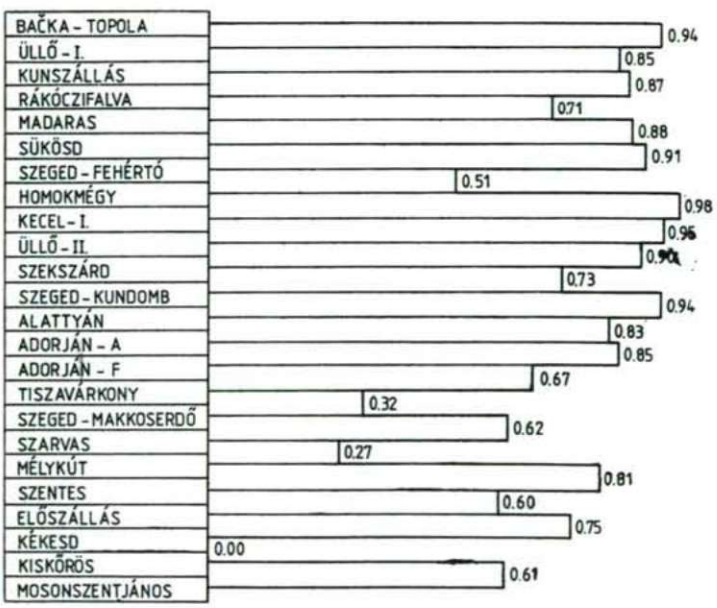


Fig. 12. Result of complex clustering on the basis of the generalized distances regarding female findings (FCR)

following sites could be linked at the 0.95 level: Homokmégy, Kecel–I, Tiszavárkony, Üllő–I and II, while the findings from Rákóczifalva entirely differ from the rest of the samples at the 0.60 level.

Fig. 10 shows the single-link clustering of the generalized distances in the case of the female series. Three sites could be linked at the 0.95 level (Homokmégy, Kecel–I, Üllő–II), while the findings from Szarvas could only be linked to every other series at the 0.65 level.

The result of the complete-link clustering in regard to the males is shown in Fig. 11. The series can practically be divided into two large groups, the boundary line is formed by the Rákóczifalva findings. Distribution into four subgroups was possible within the larger group.

Fig. 12 demonstrates the female series in a similar manner. Here, the Mosonszentjános series is completely separated, the rest of the finding groups are in relationship with each other to a lower or higher degree and form a connected group. Nevertheless, at least six subunits can be separated within this large group.

Table 1. Parameters of the combined Avar Period sample

Males				Females			
No. Martin	*N*	$\bar{X}$	*S*	No. Martin	*N*	$\bar{X}$	*S*
1.	724	182.45	6.83	1.	611	174.68	6.05
8.	724	144.31	6.45	8.	641	140.42	5.97
9.	786	97.11	4.61	9.	697	93.99	4.28
17.	513	131.21	5.92	17.	475	125.30	5.60
38.	407	1421.00	115.16	38.	365	1288.70	105.98
45.	515	134.93	5.78	45.	476	126.13	5.59
47.	498	119.60	6.84	47.	428	111.72	6.21
48.	658	70.98	4.65	48.	589	67.17	4.67
51.	691	40.61	1.94	51.	630	39.32	1.81
52.	695	33.69	2.38	52.	636	33.51	2.02
54.	650	25.75	1.93	54.	578	24.72	1.99
55.	675	51.95	3.71	55.	605	49.44	3.24
66.	573	102.44	6.52	66.	521	93.90	6.05
69.	670	33.13	3.33	69.	595	29.78	3.74

Discussion

The 77% significant deviation of the male and female standard deviation from the HOWELLS-type mean-sigmas according to character of the combined samples unambigously proves that the comparison of the Avar-Period series — with comprising Mongoloid elements — is more appropriate with the weighted standard deviation values obtained on the basis of combining several Avar-Period series. Since the significant deviations were experienced in 81% in the case of males, and in 61% in the case of females, it is also more appropriate to calculate the weighted standard deviations for the male and female types separately, and then count with these values later on.

Table 2. Comparison of the HOWELLS-type mean sigmas and the standard deviation of the Avar sample

No. Martin	Mean sigmas (Howells)	Males			Females		
		S Avars	Diff.	Signifi-cance	S Avars	Diff.	Signifi-cance
1.	6.09	6.83	4.64	++	6.05	−0.59	−
8.	5.03	6.45	10.76	++	5.97	6.71	++
9.	4.32	4.61	2.69	+	4.28	−0.33	−
17.	5.12	5.92	5.02	++	5.60	2.90	+
45.	5.10	5.78	4.29	++	5.59	2.98	+
47.	6.33	6.84	2.56	+	6.21	−0.54	−
48.	4.28	4.65	3.15	++	4.67	3.14	++
51.	1.82	1.94	2.48	+	1.81	−0.17	−
52.	2.01	2.38	6.88	++	2.02	0.19	−
54.	1.81	1.93	2.38	+	1.99	3.37	++
55.	3.03	3.71	8.26	++	3.24	2.43	+
66.	6.62	6,52	−0.50	−	6.05	−2.76	+
69.	2.84	3.33	6.32	++	3.74	10.94	++

Remark to the significance level:
++ = highly significant + = significant − = non significant

The PENROSE-type distances provide more exact comparison.

However, depending on the distance taken as the base for clustering (of size, shape, or generalized), the degree of similarity to one another of the various series may also differ.

Despite these, we succeeded in finding such series, which on the basis of all three distances could be linked to each other at the same level.

Therefore, in the case of males, linkage at a level of 0.95% was found for all three distances, thus from the studied series, the Homokmégy and Kecel–I groups of findings were those being the most similar to each other. The findings from Madaras and Szarvas, Szekszárd and Előszállás, Kékesd occur together in all three cases. The series from Kunszállás and Mosonszentjános could be linked to each other more strongly, although their similarity to the rest of the samples is slight. Eventually, the male findings excavated at Rákóczifalva unambigously show the least similarity to every other sample.

In the case of females the findings from Homokmégy, Kecel–I and Üllő–II show the strongest linkage to each other regarding all three distances. The findings from Sükösd and Szeged–Fehértó, Madaras and Mélykút, Tiszavárkony and Adorján–A, Előszállás and Kékesd also occur together. The series belonging to the Szarvas site was the most different from the rest.

Both in the case of males and females, the group of findings from Homokmégy and Kecel–I, Előszállás and Kékesd were the most similar to each other. Therefore, sex differences could not be determined from these. At the same time, however, in the rest of the samples, various groupings were possible according to the distances calculated on the basis of the measurements of the male and female findings.

The groups received with clustering cannot be attached to geographical regions, since the connection of the series from Kunszállás and Mosonszentjános in the case of males; and the series from Tiszavárkony and Adorján in the case of females could be determined besides great, different geographical distances.

At the same time it seems that the connection of the series independent of the geographical situation.

The demonstrated results are also dependent on many collateral facts, like the degree of cemetery excavation (partial or complete), the condition of preservation of the findings, etc. Therefore, our aim could only be stricted to attempt the outlining of the relationships between certain series on a more exact base, making use of the opportunity proved by the computer — by no means with the demand to also expand the connections between the various groups of findings (population units) to genetic, ethnic, or other relationships.

References

BARTUCZ, L. and FARKAS, GY. (1957): Zwei Adorjáner Gräberfelder der Awarenzeit aus anthropologischem Gesichtspunkte betrachtet. — Acta Biol. Szeged. *3*, 315–347.

K. ÉRY, K. (1968): Magyarország közzétett történeti embertani leletei (Hungary's published historical anthropological finds). — Anthrop. Közl. *12*, 173–196.

K. ÉRY, K. (1970): Összehasonlító biometriai vizsgálatok VI–XII. századi Közép-Duna medencei népességek között (Comparative biometrical examinations in 6th–12th century populations of the Middle-Danubian basin). — Anthrop. Közl. *14*, 7–34.

FARKAS, GY. and MARCSIK, A. (1984): Anthropological findings of Avar-Period from the Bačka-Topola (Yugoslavia) (Publication of data) — Acta Biol. Szeged. *30*, 191–205.

KŐHEGYI, M. and MARCSIK, A. (1971): The Avar Age cemetery at Sükösd. — Acta Ant. et Arch. Szeged. *14*, 87–94.

KNUSSMANN, R. (1967): PENROSE-Abstand und Diskriminalanalyse. — Homo. *18*, 134–140.

LIPTÁK, P. (1951): Étude anthropologique du cimetière avare d'Áporkaiürbőpuszta (Commune de Bugyi). — Ann. Hist. — nat. Mus. Nat. Hung. *1*, 232–259.

LIPTÁK, P. (1954): Kecel-környéki avarok (Les averes des environs de Kecel). — Biol. Közl. *2*, 159–180.

LIPTÁK, P. (1955): Recherches anthropologiques sur les ossements avares des environs d'Üllő. — Acta Arch. Hung. *6*, 231–316.

LIPTÁK, P. (1956): Contributions à l'anthropologique des temps avars de la région de Kiskörös. — Crania Hung. *1*, 47–52.

LIPTÁK, P. (1957): Homokmégy-Halom avarkori népessége (La population de Homokmégy-Halom dans l'époque des avars). — Anthrop. Kölz. *4*, 25–42.

LIPTÁK, P. (1958): Awaren und Magyaren im Donau-Theiss Zwischenstromgebiet. — Acta Arch. Hung. *8*, 199–268.

LIPTÁK, P. (1959): The "Avar Period" Mongoloids in Hungary. — Acta Arch. Hung. *10*, 251–279.

LIPTÁK, P. (1974): Anthropological analysis of the Avar-Period population of Szekszárd-Palánk-puszta. — Acta Biol. Szeged. *20*, 199–211.

LIPTÁK, P. and MARCSIK, A. (1966): Szeged-Kundomb avarkori népességének embertani vizsgálata (Die anthropologische Untersuchung des Gräberfeldes Szeged-Kundomb aus der Awarenperiode). — Anthrop, Közl. *10*, 13–56.

LIPTÁK, P. and MARCSIK, A. (1971): Anthropological investigation of the cemeteries from the 10th and 10th–11th centuries, excavated at Szarvas. — Acta Biol. Szeged. *17*, 209–221.

LIPTÁK, P. and MARCSIK, A. (1975): Skeletal remains of the Avar-Period and 10th century cemetery excavated at Rákóczifalva-Kastélydomb. — Acta Biol. Szeged, *21*, 165–179.

LIPTÁK, P. and MARCSIK, A. (1976): A Madaras-Téglavető avar temető csontvázmaradványainak embertani jellemzése (Anthropologische Charakteristik der Skelettreste aus dem awarischen Gräberfeld bei Madaras-Téglavető). — Cumania. *4*, 115–140.

LIPTÁK, P. and VARGA, I. (1971): Charakterisierung des anthropologischen Materials des awarischen Gräberfeldes von Kunszállás. — Móra Ferenc Múz. Évk. 1971/72, 71–79.

LIPTÁK, P. and VÁMOS, K. (1969): A "Fehértó-A" megnevezésű avarkori temető csontvázanyagának embertani vizsgálata (Anthropologische Untersuchung des Skelettmaterials des awarenzeitlichen Gräberfeldes von "Fehértó-A". — Anthrop. Közl. *13*, 3–30.

MARCSIK, A. (1971): A mélykúti avarkori temető embertani leleteinek vizsgálata (Anthropological investigation of a cemetery at Mélykút from the Avar-Period). — Anthrop. Közl. *15*, 87–95.
MARTIN, R. and SALLER, K. (1956): Lehrbuch der Anthropologie. Bd. 1. Stuttgart. 429–500.
NEMESKÉRI, J. (1943): A győri avar temető koponyáinak antropologiai vizsgálata (Anthropologic studies on the skulls from the Avar Age cemetery at Győr). — Győr története (The History of Győr). *3*, 61–71.
THOMA, A. (1957): Folytonos eloszlású jellegek variációjának mérése (The measurement of the variation of characteristics with continuous distribution), — Anthrop. Közl. *4*, 67–79.
TÓTH, T. (1962): Le cimetière de Csákberény provenant des débuts de l'époque avare (VI^e at VII^e siécles). Esquisse paléoanthropologique. — Ann. Hist.-nat. Mus. Nat. Hung. *54*, 521–549.
VÁMOS, K. (1973): „Szeged-Makkoserdő" avarkori népességének embertani vizsgálata (Die anthropologische Untersuchung der awarenzeitlichen Bevölkerung von „Szeged-Makkoserdő)". — Anthrop, Közl. *17*, 29–39.
WENGER, S. (1953): L'anthropologique du cimentière de Jánoshida-Tótkérpuszta. — Ann. Hist.-nat. Mus. Nat. Hung. *4*, 231–244.
WENGER, S. (1955): Szentes-Kaján népvándorláskori népességének embertani típusai (VII–VIII. szd.) (Types anthropologiques de la population Szentes-Kaján provenant VII–VIII^e siècles). — Ann. Hist.-nat. Mus. Nat. Hung. *6*, 391–410.
WENGER, S. (1957): Données ostéométriques sur le matériel anthropologique du cimetière d'Alattyán-Tulát, provenant de l'époque avare. — Crania Hung. *2*, 1–55.
WENGER, S. (1967): Adatok az avarkor népességének antropológiájához (Data to the anthropology of the population in the Avar Age). — Anthrop. Közl. *11*, 199–215.

Adress of the authors:
DR. GY. FARKAS
Department of Anthropology,
A. J. University, H-6701 Szeged,
P.O. Bo x 660, Hungary
DR. P. HUNYA
Kalmár Laboratory of Cybernetics,
A. J. University, H-6720 Szeged,
P.O. Box 652, Hungary

Acta Biol. Szeged, 30, pp. 191–205 (1984)

AVAR PERIOD ANTHROPOLOGIC FINDINGS FROM BAČKA-TOPOLA SITE (YUGOSLAVIA) (PUBLICATION OF DATA)

GY. FARKAS AND ANTONIA MARCSIK

Attila József University, Department of Anthropology, Szeged

(Received June 30, 1983)

Abstract

The main characteristics of 49 Avar-Age male and 52 female skeletons excataved at the Bačka-Topola-Bánkert site (Yugoslavia), an area extremely rich in archeological furniture, are reviewed. Some of the skeletons are of Mongolid character.

In the same area Sarmatian graves were also found. The occurrence of skeletons of Mongolid character in these graves points to Sarmatian-Avar relationships.

A detailed evaluation of the skeletons from the paleopathological viewpoint has already been published. The publications are listed under References.

Key-words: Avar Age, Mongolid character, Bačka-Topola site.

Introduction

The archeologists of the Subotica City Museum, with the leadership of LÁSZLÓ SZEKERES, archeologist, excavated a total of 220 graves between 1958–1977 in Yugoslavia in the Bačka–Topola–Bànkert area, which is alongside the highway leading from Subotica to Novi Sad (SZEKERES, 1978, VÉKONY, 1983).

For the present evaluation the graves No. 1–196 were at our disposal.

The findings can be divided into two archeological periods: Sarmatians from the 3rd and 4th centuries and Avars from the 6th to 8th centuries.

There were no skeletons in five Avar graves (Nos 19, 53, 56, 85, 185) and in six Sarmatian graves (Nos 4, 37, 67, 81, 115, 132). At the same time, there had been dual buryings in 11 Avar graves (Nos 18, 57, 86, 96, 124, 129, 149, 165, 188, 191, 194), and in one Sarmatian grave (No. 127). Further two findings (one of which was Mongolid (had been excavated in 1958 (FARKAS, 1971).

Numbered till No. 196 — also taking into account the dual buryings (dual graves = 12, and two graves from 1958) — the total number of graves was 210. From these the number of graves containing skeletons from the Avar Age was 166, and those containing skeletons from the Sarmatian Age was 33. In the present paper we deal with the findings from the Avar Age — with the exception of the two Avar skulls excavated in 1958. The graves No. 197–220 excavated in 1977 were not at our disposal.

Methods

Table 1 gives information on the distribution of the excavated findings by sex, age of death and archeological period. To decide the sex, 22 characters were considered; the age of death was determined on the basis of the morphological characteristics of the sutures, of the teeth and of the facies symphyseos.

According to the method of MARTIN (MARTIN and SALLER 1956), 9 neurocranium and 17 splanchnocranium measurements were taken and six morphological characteristics were considered; furthermore, with the method of BREITINGER and BACH (BREITINGER, 1937, BACH, 1965) the stature values were calculated from the measurements of the long bones. These individual values for adults are presented in Tables 4 and 5 and those for the fragmentary material are given in the text.

Tables 2 and 3 comparise come important parameters of the characteristics and indices regarding both sexes (sample size, range, arithmetic mean, standard deviation). The calculations were carried out with a table calculator.

Results

From the findings 57 were males, 65 were females and 40 (24%) were children. The sex could not be determined in two cases.

As to the age of death the male findings were almost equally disrtibuted in the age groups adultus, maturus and senilis, whereas a conspicuously high proportion of the female ones (49%) died while being "adulta" i. e. 25–40 years old.

This result is consistent with the experiences gained from other Avar-Period cemeteries.

The data are presented in Tables 2 and 3, the two sexes may be characterized in the following:

Males: the neurocranium is medium long, moderate wide, low, medium high; the brain capacity is medium, the base of the skull is short and medium long; the forehead is narrow and medium wide. On the basis of the indices the neurocranium is short (brachycranic), its height is meduim (orthocranic); the forehead is narrow (stenometopic). From norma verticalis the most frequent are the pentagonoid and sphenoid forms, the glabella is mainly of grade 3–4; the protuberantia occipitalis externa is of grade 3.

According to the absolute measurements the splanchnocranium is moderately wide and high; the upper face is medium high and high; the orbit is narrow and high; the nasal cavity is wide and high and very high; the palate is medium long and medium wide; the mandible is medium wide and wide. The face is straight (orthognathous). On the basis of the indices, the face is narrow (leptoprosopic), the upper face is narrow and medium wide (leptene and mesene); the orbit is high (hypsiconchous); the nasal cavity is medium wide (mesorrhine); the palate is very short (hyperbrachystaphyline). The fossa canina is flat, but is completely filled up in many cases (23.5%); the spina nasalis anterior is mainly of grade 2; the alveolar prognathism is mostly moderate.

The males are of tall-medium stature according to the stature mean, on the basis of the frequency of the stature categories they are mainly of medium stature. The difference in stature between the two sex is 7.7 cm, in favour of the males, on the basis of the means.

Females: their neurocrania are medium long, medium wide, low and medium high, according to the absolute measurements. Their brain capacities are medium and large, their foreheads are narrow and medium wide. According to the indices

they are short (brachycranic), medium high and low (orthocranic and chamaecranic); the forehead are medium wide (metriometopic). From norma verticalis the most frequent is the pentagonid contour, the glabella is firstly of grade 2, and the protuberantia occipitalis externa is of grade 0.

According to the absolute measurements, the splanchnocranium is medium wide and high; the upper face is high; the orbit is narrow and medium high; the nasal cavity is medium wide and high; the palate is medium long and short, medium wide and narrow; the mandible is medium wide; the face is straight (orthognathous). On the basis of the indices, the splanchnocranium is narrow (leptoprosopic); the upper face is medium wide and narrow (mesene and leptene); the orbit is high (hypsiconchous); the nasal cavity is wide (chamaerrhine); the palate is wide (hyperbrachystaphyline). The fossa canina is filled up and flat; the spina nasalis anterior is mainly of grade 2; the alveolar prognathism is moderate.

On the basis of the stature mean and also according to the distribution in the stature categories, the female were of tall stature.

The general characterization presented above shows no basic differences between the female and the male skeletons.

The taxonomic analysis of the findings will soon be reported.

Tables 4 and 5 demonstrate the individual measurements and characteristics of the well-preserved findings. The appropriate data of the fragmentary findings are given in the following:

Males:

Grave 7. — Sexual index: +1.7. Stature: 163.5.
Grave 28. — G-op: 186, m-op: 180, n-ba: 92, eu-eu: 146, ft-ft: 102, po-b: 113. Juv. Sexual index: +1.0. N. vert.: pent. Glabella: 3, prot. occ. ext.: 0, stature: 168,7.
Grave 31. — Ad.-Mat. Sexual index: +1.3.
Grave 68. — Id-gn: 32, width of ramus mandibulae: 35. Ad. Sexual index: +1.2. N. vert.: plag. Prot. occ. ext.: 1.
Grave 86a. — G-op: 185, m-op: 187, eu-eu: 144, id-gn: 30, height of ramus mandibulae: 56, width of ramus mandibulae: 30. Mat. Sexual index: +0.7, N. vert.: plag. Prot. occ. ext.: 3. Stature: 163,9.
Grave 109. — Width of nasal cavity: 28, ol-sta: 43, enm-enm: 43, height of ramus mandibulae: 60, width of ramus mandibulae: 33. Ad. Sexual index: 0. Glabella: 1, prot. occ. ext.: 0, fossa canina: 2, sp. n. ant.: 2, alv. progn.: 2, stature: 169.3.
Grave 118. — Eu-eu: 153, go-go: 96, height of ramus mandibulae: 63, width of ramus mandibulae: 31. Juv. Sexual index: +1.1. Prot. occ. ext.: 1, stature: 173.8.
Grave 149a. — Mat. Sexual index: +1.2. Glabella: 0.
Grave 162. — Go-go: 103, Juv. Sexual index: +2,0.

Females:

Grave 2. — Id-gn: 27. Ad. Sexual index: −2.0, Glabella: 1.
Grave 8. — Ad.-Mat. Sexual index: −0.6. Glabella: 1.

Grave 15. — G-op: 190, m-op: 187. eu-eu: 146, po-b: 112, height of ramus mandibulae: 66, width of ramus mandibulae: 38, Ad. Sexual index: −1.5. N. vert.: pentagonoid, Glabella: 1, prot. occ. ext.: 0.

Grave 22. — G-op: 187, m-op: 185, eu-eu: 124, ft-ft: 91, id-gn: 31. Sen. Sexual index: −0.7. N. vert.: ell. Glabella: 1, prot. occ. ext.: 0, stature: 163.3.

Grave 42. — Eu-eu: 152, ft-ft: 98, po-b: 110. Sen. Sexual index: −1.3. N. vert.: plag. Stature: 161,1.

Grave 74. — G-op: 181, m-op: 178, eu-eu: 143, ba-b: 107, id-gn: 24, height of ramus mandibulae: 63, width of ramus mandibulae: 30. Sen. Sexual index: −0.9. N. vert.: pent. Prot. occ. ext.: O, stature: 155,6.

Grave 134. — Eu-eu: 129, go-go: 88, height of ramus mandibulae: 52, width of ramus mandibulae: 27. Ad. Sexual index: −1.6. N. vert.: plag. Prot. occ. ext.: 0, stature: 158,9.

Grave 139. — Ft-ft: 90, width of ramus mandibulae: 28. Sen. Sexual index: −1.3, N. vert.: sphen. Glabella: 3.

Grave 153. — G-op: 174, m-op: 174, eu-eu: 146, ft-ft: 99, ba-b: 100, width of ramus mandibulae: 28, Ad. Sexual index: −1.1. N. vert.: ell. Glabella: 1, prot. occ. ext.: 0, alv. progn.: 2, stature: 157,6.

Grave 177. — G-op: 191, eu-eu: 133, ft-ft: 91, ol-sta: 41, id-gn: 33, height of ramus mandibulae: 66. Mat. Sexual index: −0.2. N. vert.: plag. Glabella: 1, prot. occ. ext.: 0, alv. progn.: 3, stature: 155.7.

Grave 182. — Go-go: 96. Ad. Prot. occ. ext.: 0.

Grave 190. — Eu-eu: 130. Ad. Sexual index: −1.0. Prot. occ. ext.: 0.

Table I. Distribution of findings by age of death, sex and archeological periods

	Age of death	Inf. I. II.		Males		Females		Underfinable		Total	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%
Avars	Infantia I.	12	30.0	—	—	—	—	—	—	12	7.3
	Infantia II.	28	70.0	—	—	—	—	—	—	28	17.1
	Juvenis	—	—	4	7.0	7	10.8	1	50.0	12	7.3
	Adultus	—	—	15	26.3	32	49.2	—	—	47	28.6
	Maturus	—	—	15	26.3	12	18.4	—	—	27	16.5
	Senilis	—	—	18	31.6	12	18.4	—	—	30	18.3
	Unknown	—	—	5	8.8	2	3.1	1	50.0	8	4.9
	Together	40		57		65		2		164	
Sarmatians	Infantia I.	1	20.0	—	—	—	—	—	—	1	3.0
	Infantia II.	4	80.0	—	—	—	—	—	—	4	12.1
	Juvenis	—	—	1	6.3	—	—	—	—	1	3.0
	Adultus	—	—	6	37.5	4	66.7	3	50.0	13	39.4
	Maturus	—	—	3	18.7	1	16.6	—	—	4	12.1
	Senilis	—	—	5	31.2	—	—	—	—	5	15.2
	Unknown	—	—	1	6.3	1	16.6	3	50.0	5	15.2
	Together	5		16		6		6		33	

Table 2. Parameters of some important measurements and indices for Avar males

No. (Martin)	*n*	*w*	$\bar{X}$	*s*
1.	42	167–200	181.95	6.54
1c.	41	163–188	178.29	6.44
5.	29	86–111	96.83	5.26
8.	43	135–155	145.58	4.54
9.	42	82–103	95.67	4.30
17.	30	116–142	129.20	6.09
20.	36	106–122	114.06	3.79
32/la.	31	41–51	47.42	2.66
38.	30	1214–1580	1405.90	89.29
40.	26	85–107	93.73	6.16
45.	32	124–146	133.94	5.84
46.	34	87–113	99.65	5.66
47.	33	112–140	124.48	7.66
48.	35	65–86	74.83	5.11
51.	38	35–41	38.12	1.42
52.	38	28–47	35.40	3.41
54.	35	22–34	26.94	2.57
55.	35	48–62	53.17	3.23
62.	30	37–52	43.33	3.21
63.	35	33–44	39.34	2.82
65.	27	103–136	122.04	6.00
66.	32	92–118	102.03	5.69
69.	37	29–42	34.30	3.35
70.	38	54–81	65.97	6.72
71.	41	29–38	33.37	2.77
72.	28	78–97	85.61	4.48
Stature	44	160.1–174.0	167.16	3.28
Sex. i.	50	0–+2	0.96	0.46
5:8	29	57–82	66.48	5.10
20:1	36	55–70	62.83	2.52
20:8	36	72–88	78.28	4.10
66:45	25	70–86	75.84	4.20
45:8	32	85–99	91.94	3.82
9:45	32	65–82	71.50	3.35
8:1	42	68–88	80.05	4.05
17:1	30	67–77	71.10	2.93
17:8	30	80–104	88.63	5.89
9:8	41	58–75	65.85	3.11
47:45	28	79–103	92.43	5.43
48:45	30	46–63	55.57	3.57
52:51	38	76–124	90.45	8.62
54:55	34	42–61	50.68	5.07
63:62	29	75–105	90.93	7.28

Table 3. Parameters of some important measurements and indices for Avar females

No. (Martin)	*n*	*w*	$\bar{X}$	*s*
1.	39	161–191	176.51	7.00
1c.	39	161–187	173.95	6.47
5.	26	85–110	95.46	5.69
8.	44	124–154	140.43	7.41
9.	42	85–102	92.71	3.95
17.	27	114–142	124.93	6.54
20.	35	100–119	109.49	4.83
32/la.	25	42–54	47.76	3.18
38.	26	1134–1516	1307.00	109.30
40.	20	82–108	92.60	7.01
45.	29	110–144	126.10	7.10
46.	31	88–109	95.39	5.49
47.	30	101–130	114.30	7.08
48.	29	62–78	70.28	4.14
51.	34	34–44	37.41	1.96
52.	33	29–38	33.61	2.18
54.	29	22–30	25.59	2.24
55.	30	51–57	50.26	2.93
62.	29	37–63	42.45	5.17
63.	28	33–49	38.50	3.38
65.	27	108–133	118.52	8.11
66.	30	85–114	96.20	6.32
69.	40	20–37	30.10	3.90
70.	38	51–72	60.42	4.75
71.	42	26–41	32.00	3.53
72.	22	79–93	85.55	4.11
Stature	39	153.1–167.6	159.45	3.01
Sex. i.	52	0–−2	−1.07	0.47
5:8	25	58–83	67.72	5.36
20:1	32	57–68	62.19	2.69
20:8	34	72–90	77.74	4.21
66:45	21	65–87	76.91	5.25
45:8	27	73–97	89.15	5.27
9:45	28	65–82	73.61	3.65
8:1	38	66–93	79.90	5.03
17:1	25	65–77	71.24	2.88
17:8	26	79–108	89.04	6.81
9:8	37	57–73	65.81	3.55
47:45	25	81–101	90.60	3.55
48:45	25	50–61	55.40	3.30
52:51	32	81–112	90.38	6.51
54:55	27	39–61	50.74	5.33
63:62	25	65–129	90.64	12.70

Table 4. Individual measurements and morphological characters — Avar males

No. (MARTIN)	No. of graves									
	9.	18a.	20.	25.	33.	34.	36.	41.	46.	48.
1.	184	—	173	177	181	184	200	189	183	184
1c.	183	—	—	169	174	185	188	182	184	181
5.	—	—	—	90	99	95	111	94	—	99
8.	148	—	143	149	152	147	136	152	149	149
9.	97	96	100	92	99	103	91	99	98	96
17.	—	—	—	124	129	126	135	126	131	130
20.	117	—	—	111	113	114	118	111	110	117
32/1a.	50	—	—	45	41	46	—	48	—	50
38.	—	—	—	1338	1321	1458	1434	1444	1512	1476
40.	—	—	—	95	87	89	—	95	—	99
45.	—	—	—	135	—	144	—	137	—	143
46.	—	104	97	99	113	103	—	101	—	105
47	—	137	140	122	133	130	—	127	—	128
48.	—	83	86	74	80	77	—	75	—	81
51.	40	40	37	38	41	39	—	37	—	39
52.	38	36	39	35	38	35	—	32	—	35
54.	—	28	27	29	34	28	—	28	—	30
55.	—	58	62	53	58	52	—	51	—	54
62.	48	41	—	41	—	—	—	45	—	37
63.	41	41	—	37	43	38	35	42	—	—
65.	128	124	—	122	124	124	—	—	—	123
66.	106	98	—	105	103	110	—	101	110	108
69.	34	37	—	37	35	37	39	36	—	37
70.	72	69	—	68	71	63	69	72	—	57
71.	37	37	—	34	33	37	37	32	33	38
72.	—	—	—	78	—	82	—	83	—	82
Age Sex. index	Ad. +0.7	Mat. +0.6	Mat.-Sen. +1.4	Sen. +1.1	Mat. +1.1	Ad. 0.0	Mat.-Sen. +1.4	Ad. +1.5	Ad. +1.3	Sen. +1.0
N. vert.	Ovoid	—	Spher.	Romb.	Sphen.	Ell.	Plag.	Sphen.	Plag.	Ovoid
Glabella	2	3	2	3	4	2	5	4	3	3
Prot. o. ext.	1	—	—	1	1	2	4	1	—	2
Fossa can.	—	2	—	1	—	2	1	2	—	2
Sp. n. ant.	2	1	—	1	—	—	—	2	—	—
Alv. progn.	3	2	2	2	2	2	3	2	—	3
Stature	161.3	166.7	166.3	169.2	165.1	168.2	166.5	168.9	165.2	166.3

Table 4. (continuation 1)

No. (MARTIN)	No. of graves									
	58.	62.	64.	66.	75.	77.	83.	87.	93.	96b.
1.	180	180	187	167	191	181	184	187	173	186
1c.	179	174	187	163	182	177	180	184	170	183
5.	100	—	92	—	—	98	97	98	97	—
8.	147	148	147	147	147	142	150	155	142	145
9.	97	96	93	97	101	97	101	94	82	95
17.	135	127	128	—	—	134	128	132	125	—
20.	115	—	121	112	106	112	118	114	109	—
32/1a.	45	—	45	46	46	50	46	45	51	45
38.	1485	1379	1494	—	—	1414	1458	1580	1274	—
40.	94	—	86	—	—	99	98	88	94	—
45.	136	137	132	136	142	131	137	132	127	126
46.	102	—	96	102	103	105	104	99	87	97
47.	134	—	113	—	112	124	129	128	113	127
48.	80	—	73	70	65	76	73	76	68	75
51.	37	—	38	38	41	38	37	39	37	39
52.	36	—	34	37	31	33	35	35	30	35
54.	27	—	28	23	28	27	27	26	22	24
55.	56	—	55	54	50	52	53	54	48	53
62.	44	—	43	—	52	47	43	—	44	42
63.	37	—	37	43	43	39	39	34	33	37
65.	127	130	123	—	121	120	119	119	126	—
66.	108	118	106	—	105	96	98	92	100	107
69.	32	30	31	—	32	37	38	37	33	—
70.	71	70	70	—	61	73	68	63	60	58
71.	36	38	33	—	36	31	30	30	29	29
72.	87	—	87	—	80	81	86	90	86	97
Age Sex. index	Sen. +1.0	Ad. +1.5	Sen. +0.8	Ad. +0.6	Sen. +1.3	Mat.-Sen. +1.1	Mat.-Sen. +0.9	Mat. +0.2	Mat. +0.7	Mat. +1.0
N. vert.	Sphen.	Pent.	Ovoid	Sphen.	Pent.	Pent.	Pent.	Pent.	Sphen.	—
Glabella	3	4	2	3	4	4	4	4	4	4
Prot. o. ext.	3	3	2	2	3	3	—	3	3	3
Fossa can.	2	—	2	3	3	1	2	2	2	2
Sp. n. ant.	3	—	3	—	2	—	4	—	4	—
Alv. progn.	2	—	1	1	2	2	2	1	2	1
Stature	170.2	170.2	166.8	169.4	166,7	174.0	171.9	168. 0	165.7	164.1

Table 4. (continuation 2)

No. (MARTIN)	No. of graves									
	100.	101.	102.	107.	116.	120.	122.	129a.	131.	140.
1.	186	195	180	190	183	179	185	180	183	177
1c.	185	181	177	188	178	171	185	179	175	169
5.	—	—	97	108	—	101	93	86	99	—
8.	140	135	144	136	144	144	146	152	152	141
9.	93	89	89	102	91	96	92	97	99	89
17.	—	—	129	142	—	136	127	127	125	—
20.	117	—	114	120	113	118	116	115	112	—
32/1a.	50	—	48	49	48	50	50	45	—	46
38.	—	—	1388	1500	—	1396	1282	1489	1405	—
40.	—	—	85	96	—	93	86	85	107	—
45.	129	—	126	124	125	139	134	129	131	—
46.	88	97	90	90	101	98	102	104	98	97
47.	121	123	120	123	114	137	—	133	128	117
48.	70	73	71	70	67	76	80	81	82	67
51.	38	37	37	38	37	40	38	41	39	37
52.	32	36	36	34	30	33	35	38	36	28
54.	22	26	24	26	28	—	28	28	30	23
55.	52	51	49	53	48	51	57	56	57	49
62.	40	45	37	44	45	42	—	—	44	44
63.	39	41	37	36	40	41	—	39	40	37
65.	118	113	—	—	121	122	—	119	—	—
66.	97	—	94	—	104	104	—	—	—	97
69.	31	33	31	31	33	42	—	38	37	34
70.	69	69	57	76	59	71	—	68	—	61
71.	30	36	29	34	31	34	—	35	34	33
72.	90	—	92	86	81	91	93	85	—	84
Age Sex. index	Mat. 0.0	Ad. +0.7	Ad. 0.0	Sen. +1.1	Sen. +1.0	Mat. +1.2	Mat. +0.7	Ad. +1.0	Mat. +1.0	Ad. +0.7
N. vert.	Pent.	Ovoid	Pent.	Ovoid	Pent.	Sphen.	Pent.	Pent.	Ovoid	Pent.
Glabella	4	5	3	5	4	5	1	2	3	4
Prot. o. ext.	0	3	3	3	3	3	2	0	2	2
Fossa can.	2	3	1	4	2	3	—	1	1	1
Sp. n. ant.	3	4	5	4	2	2	—	—	3	—
Alv. progn.	2	2	2	1	1	3	2	3	3	2
Stature	165.5	168.2	164.0	166.5	166.8	167.1	170.0	170.9	166.1	—

Table 4. (continuation 3)

No. (MARTIN)	No. of graves									
	142.	145.	156.	157.	159.	179.	186.	194a.	194b.	196.
1.	177	183	171	172	187	178	181	173	174	179
1c.	176	182	170	169	188	174	178	172	169	178
5.	—	90	89	97	102	101	98	96	94	96
8.	148	143	145	142	139	147	142	145	144	146
9.	95	98	95	100	98	98	93	90	93	96
17.	—	127	116	133	142	134	129	120	122	120
20.	109	113	109	120	122	113	110	112	113	111
32/1a.	—	50	49	51	—	45	46	—	43	50
38.	—	1405	1251	1335	1528	1432	1379	1303	1214	1335
40.	—	85	92	91	—	101	93	98	96	103
45.	—	132	131	133	130	144	137	129	136	136
46.	—	102	94	98	—	101	96	—	100	105
47.	—	121	117	125	—	128	123	116	122	112
48.	—	73	69	79	—	77	74	73	78	69
51.	37	39	35	38	35	37	38	38	38	39
52.	29	34	33	36	29	37	32	35	47	35
54.	—	26	30	26	—	27	25	24	27	28
55.	—	53	49	55	—	54	52	55	57	49
62.	—	45	41	47	—	41	40	39	46	44
63.	—	44	37	38	—	43	40	39	43	39
65.	—	122	—	122	—	—	125	115	121	128
66.	105	96	97	—	—	103	105	96	99	98
69.	31	34	29	33	—	37	34	29	33	33
70.	56	68	54	81	—	72	76	55	65	64
71.	32	29	32	32	—	34	33	34	32	37
72.	—	86	82	86	—	88	85	—	88	80
Age Sex. index	Mat. +1.1	Sen. +1.6	Sen. 0.0	Sen. +1.6	Ad.-Mat. +1.1	Ad. +1.2	Sen. +1.0	Juv.-Ad. +0.8	Sen. +0.8	Sen. +0.5
N. vert.	Sphen.	Pent.	Sphen.	Sphen	Pent.	Sphen.	Pent.	Pent.	Sphen.	Sphen.
Glabella	4	5	3	4	5	2	4	3	3	3
Prot. o. ext.	3	1	3	5	4	1	4	0	2	3
Fossa can.	—	1	5	4	—	3	3	3	3	2
Sp. n. ant.	—	2	1	2	—	2	1	3	2	—
Alv. progn.	—	2	3	3	—	2	2	—	1	3
Stature	167.5	172.3	160.0	172.8	167.1	166.4	166.4	160.5	164.2	161.9

Table 5. Individual measurements and morphological characters — Avar females

No. (MARTIN)	No. of graves									
	3.	12.	13.	16.	21.	26.	35.	38.	45.	50.
1.	184	182	171	—	173	185	172	175	—	177
1c.	180	177	168	—	171	182	169	173	—	174
5.	93	102	—	—	94	110	96	96	—	94
8.	154	146	140	—	142	132	145	145	—	138
9.	92	94	95	88	98	89	91	90	—	94
17.	127	136	—	—	120	142	128	123	—	127
20.	119	118	110	—	115	119	110	104	—	107
32/1a.	54	50	51	—	47	—	47	51	—	48
38.	1516	1467	—	—	1240	1406	1347	1308	—	1291
40.	101	—	—	—	98	108	83	87	—	89
45.	125	—	132	—	134	119	130	127	—	125
46.	97	—	97	101	95	103	93	89	101	95
47.	126	—	120	117	112	113	120	116	127	108
48.	76	—	74	77	68	72	70	71	76	69
51.	37	38	36	34	40	35	36	37	—	37
52.	33	34	32	38	35	36	34	35	—	36
54.	26	—	27	22	24	29	23	25	30	25
55.	52	—	57	57	49	51	54	48	50	47
62.	38	—	—	—	41	43	37	40	43	39
63.	49	—	40	40	40	40	40	36	42	37
65.	127	126	129	—	124	125	112	112	—	—
66.	101	106	—	—	104	103	85	94	—	—
69.	36	—	32	34	32	32	31	29	36	30
70.	62	61	57	—	61	72	62	60	—	—
71.	36	37	30	—	35	35	33	33	—	30
72.	86	—	84	—	83	—	90	90	—	85
Age Sex. index	Ad. −0.1	Ad. −0.6	Mat. −1.1	Ad. −0.6	Ad. −1.2	Ad. −1.0	Ad. −1.5	Ad. −1.2	Ad. 0.0	Ad. −1.0
N. vert.	Romb.	Ovoid	Ovoid	—	Ovoid	Ell.	Sphen.	Ovoid	—	Pent.
Glabella	3	3	2	4	1	2	2	1	—	3
Prot. o. ext.	0	0	1	—	0	1	0	0	—	0
Fossa can.	2	1	2	2	1	1	3	2	—	2
Sp. n. ant.	—	—	2	2	2	1	—	3	1	2
Alv. progn.	3	3	3	2	2	2	3	2	2	2
Stature	164.0	158.5	161.2	160.0	160.5	158.8	158.6	163.7	—	164.7

Table 5. (continuation 1)

No. (MARTIN)	No. of graves									
	55.	59.	63.	65.	69.	71.	76.	79.	90.	91.
1.	180	177	177	179	161	179	177	176	170	165
1c.	180	175	174	176	161	175	174	176	169	165
5.	—	93	103	96	—	96	102	96	92	—
8.	146	144	148	137	150	149	137	134	144	137
9.	102	94	98	93	86	94	96	94	90	87
17.	—	120	129	123	—	128	124	127	115	—
20.	106	111	111	108	—	113	103	108	103	112
32/1a.	47	49	51	42	—	50	42	42	45	—
38.	—	1291	1427	1265	—	1405	1214	1231	1226	—
40.	—	92	92	87	—	106	92	91	—	—
45.	124	129	137	125	110	144	125	126	120	—
46.	100	90	106	92	88	—	94	93	—	—
47.	121	121	116	114	106	119	114	113	—	—
48.	71	72	71	71	67	73	68	68	—	—
51.	40	37	39	37	37	39	39	39	36	—
52.	36	35	35	34	30	33	35	34	31	—
54.	27	24	27	24	25	—	23	—	—	—
55.	50	54	52	52	49	50	49	47	—	—
62.	45	44	43	39	41	—	38	40	—	—
63.	38	37	43	33	35	—	34	—	—	—
65.	—	122	—	112	108	136	113	119	—	—
66.	96	89	—	86	—	114	101	93	—	—
69.	34	32	32	28	27	33	—	30	23	20
70.	62	60	62	61	51	58	58	60	58	63
71.	33	30	34	26	29	34	35	31	29	27
72.	81	87	92	90	—	80	88	84	—	—
Age Sex. index	Juv.-Ad. −1.8	Sen. −1.2	Mat. −1.0	Ad. −1.2	Juv. −1.4	Mat. −0.9	Ad. −0.4	Ad. −0.9	Sen. −1.2	Mat. −1.0
N. vert.	Sphen.	Pent.	Pent.	Pent.	Plag.	Plag.	Pent.	Pent.	Sphen.	Pent.
Glabella	1	2	1	2	1	2	3	3	2	4
Prot. o. ext.	0	0	0	0	1	2	3	0	2	0
Fossa can.	1	2	1	1	1	2	4	1	3	—
Sp. n. ant.	—	3	1	2	2	2	—	1	—	—
Alv. prog.	2	3	3	2	3	2	2	2	—	—
Stature	—	159.3	160.4	157.4	160.3	159.7	158.4	160.4	158,1	—

Table 5. (continuation 2)

No. (MARTIN)	No. of graves									
	98.	104.	114.	117.	121.	124b.	133.	138.	150.	152.
1.	169	169	182	178	—	177	—	168	—	182
1c.	166	168	181	176	—	176	167	164	—	183
5.	85	87	103	—	—	85	—	92	—	94
8.	144	142	149	145	—	146	128	142	136	125
9.	96	88	93	98	95	96	—	90	—	89
17.	114	121	133	—	—	123	120	116	—	132
20.	105	108	115	—	—	110	107	105	—	109
32/1a.	47	48	—	—	—	—	—	46	—	45
38.	1192	1251	1503	—	—	1344	—	1176	—	1249
40.	—	82	—	—	—	—	—	89	—	92
45.	130	115	134	—	121	—	—	123	—	114
46.	100	92	90	—	90	—	—	89	101	88
47.	102	111	113	—	112	—	—	101	118	110
48.	—	64	72	—	68	—	—	62	72	68
51.	37	36	36	—	36	—	—	38	40	36
52.	34	29	33	—	36	—	—	33	36	33
54.	27	26	—	26	27	—	—	23	29	24
55.	51	46	—	—	51	—	—	47	52	49
62.	—	63	—	41	—	—	—	42	53	44
63.	39	41	—	37	—	—	—	36	37	38
65.	122	111	127	111	—	119	—	111	117	111
66.	92	94	99	93	95	91	—	92	91	94
69.	25	26	28	32	28	29	27	26	34	27
70.	59	64	61	51	52	63	61	60	64	61
71.	34	28	30	28	26	35	31	35	37	31
72.	—	89	—	—	—	—	—	83	—	81
Age Sex. index	Ad. −1.1	Sen. −0.7	Sen. −0.3	Juv.-Ad. −1.1	Mat. —1.3	Ad.Mat. −1.0	Mat. −1.6	Ad. —1.6	Ad.-Sen. −0.3	Ad. −1.6
N. vert.	Pent.	Pent.	Pent.	Pent.	Plag.	Ovoid	Pent.	Pent.	—	Pent.
Glabella	2	2	2	2	1	2	—	2	2	2
Prot. o. ext.	2	0	1	0	0	1	0	0	0	0
Fossa can.	1	1	—	4	1	—	—	2	3	2
Sp. n. ant.	—	—	—	3	4	—	—	3	—	2
Alv. progn.	—	2	3	2	2	—	—	2	3	3
Stature	—	161.8	164.4	160.9	157.3	156.3	156.6	—	—	157.5

Table 5. (continuation 3)

No. (MARTIN)	No. of graves									
	153.	161.	165a.	169.	174.	180.	187.	188a.	188b.	191a.
1.	186	173	172	168	176	—	179	184	—	164
1c.	182	174	172	165	178	—	179	180	—	163
5.	96	—	—	94	97	—	95	—	—	90
8.	149	132	136	135	134	—	148	148	—	138
9.	97	89	92	86	91	94	93	97	—	85
17.	131	—	—	120	123	—	117	—	—	126
20.	118	—	111	101	108	—	108	—	—	108
32/1a.	50	—	52	45	46	—	—	—	—	49
38.	1494	—	—	1134	1214	—	1335	—	—	1191
40.	91	—	—	94	86	—	—	—	—	91
45.	133	—	124	126	130	—	—	126	—	120
46.	98	—	96	98	97	91	—	—	96	88
47.	130	—	101	110	—	—	—	—	115	109
48.	78	—	62	68	—	73	—	—	—	64
51.	39	—	34	36	37	39	—	37	44	37
52.	36	—	30	34	33	34	—	30	—	30
54.	24	—	24	28	24	28	—	—	—	22
55.	52	—	46	46	52	51	—	—	—	48
62.	45	—	38	44	38	43	37	—	44	42
63.	33	—	38	42	38	—	—	—	36	37
65.	137	113	112	112	—	—	—	—	—	112
66.	104	95	96	95	—	—	—	—	101	101
69.	37	34	28	28	—	36	—	—	35	28
70.	69	56	54	63	—	—	—	—	67	54
71.	32	31	31	30	—	31	—	35	41	33
72.	86	—	87	79	93	—	—	—	—	79
Age Sex. index	Mat. −0.1	Ad.-Mat. −1.7	Ad. −1.5	Sen. −1.4	Sen. −1.2	Ad. −1.5	Ad. −1.2	Mat. −0.7	Ad. −1.5	Sen. −1.6
N. vert.	Ovoid	Pent.	Pent.	Pent.	Pent.	—	Pent.	Ovoid	Plag.	Pent.
Glabella	2	1	1	2	2	1	2	4	1	2
Prot. o. ext.	2	0	0	0	4	—	0	5	0	1
Fossa can.	2	3	3	3	1	—	—	—	—	3
Sp. n. ant.	3	—	2	1	3	—	—	—	2	3
Alv. progn.	3	2	2	2	2	3	2	—	3	3
Stature	167.5	160.0	154.8	157.7	161.8	153.1	160.7	158.3	—	154.5

References

BACH, H. (1965): Berechnung der Körperhöhe aus den langen Gliedmassenknochen weiblicher Skelette. — Anthr. Anzeiger. *29*, 12–21.
BREITINGER, E. (1938): Zur Berechnung der Körperhöhe aus den langen Gliedmassenknochen. — Anthr. Anzeiger. *14*, 249–274.
FARKAS, GY. (1971): Anthropologische Funde von der Woiwodina. — MFMÉ, *2*, 209–212.
FARKAS, GY. and MARCSIK, A. (1979): Paläopathologische Fälle in der awarenzeitlichen Serie von Bačka-Topola, SFR Jugoslawien. — Ethnogr. — Archäol. Z. *20*, 15–33.
FARKAS, GY., MARCSIK, A. and VÉKONY, L. (1976): Vertebral deformation in the avar skeletal material. — Anthropologie (ČSSR). *14*, 231–233.
MARTIN, R. and SALLER, K. (1956): Lehrbuch der Anthropologie. — Gustav Fischer Verlag, Stuttgart.
SZEKERES, L. (1978): Necropolis from the avar period at Bačka-Topola. — Novi Sad, 1978. 157–160.
VÉKONY, L. (1983): A topolyai avar temető paleopatológiai elemzése. — Létünk. *1*, 91–98.

Address of the authors:
DR. GY. FARKAS
DR. ANTÓNIA MARCSIK
Department of Anthropology
Attila József University
H-6701 Szeged
Egyetem u. 2.
P.O. Box 660

Acta Biol. Szeged. 30, pp. 207–210 (1984)

CHRONICLE
1983

PERSONALIA

Jubilee

On November 20, 1983, DR. AMBRUS ÁBRAHÁM, academician, Kossuth Prize winner, emeritus Professor and Director of the Department of Zoology, celebrated his 90. birthday. He was awarded the Standard Order of the Hungarian People's Republic by the Presidium of the Hungarian People's Republic for his outstanding merits achieved in the field of his educational and research activities for over several decades. The Hungarian Academy of Sciences and the leaders of the county offered him their congratulations on this occasion. At the special session organized by the

Department of Zoology and the Center in Szeged of the Hungarian Society of Biology (December 8, 1983), the former students and co-workers of DR. AMBRUS ÁBRAHÁM, academician, expressed their esteem to him with their lectures. At this session, DR. IMRE TÖRŐ, academician, President of the Hungarian Society of Biology, handed over the greatest Hungarian Biologist Medal, the GELEI Medallion, to the person fêted.

Necrologue

PROF. DR. PÁL GREGUSS (1889–1984)

DR. PÁL GREGUSS retired professor died at the age of 95 on 23rd March, 1984. With his death we lost one of the greatest Hungarian botanic-anatomists.

Professor GREGUSS was the head of the Department of Botany and the director of the Botanical Garden in Szeged for 25 years.

He was born in Tornya, in the County of Arad, on 31st December, in 1889. After attending school in Arad he began his university studies in Budapest, where he qualified as a teacher of biology and chemistry.

During World War I. he worked as a soldier at the University in Prague. After returning home, he taught at a Teachers' Training College. He acquired the title of university doctor in Budapest in 1919. He was one of the assistents of Professor F. HOLLENDONNER at the Teachers' Training College in 1920. He was appointed honorary lecturer at the PÉTER PÁZMÁNY University in 1927. In the same year he was invited to organize and direct the Department of Botany at Debrecen University. A year later he was appointed professor at the Teachers' Training College in Szeged. After the death of I. GYÖRFFY he was appointed professor in the Department of Botany at Szeged university in 1940. Here he worked with inexhaustible energy, and directed the teaching and research work for 25 years.

He had an extraordinary memory and constitution. Even 2 days before his death he was in the Department in his small room, where full of plans and conception, he was still working. He dealt with almost all branches of botany (Palynology, morphology, histology, xylotomy, genetic, taxonomy, etc.).

His first article was published at the age of 20, in 1909. As a result of 75 years of hard, persistent research work he published 46 books (handbooks and textbooks), 10 university text-books and 254 articles (scientific, educational etc.).

He continued his research work even after his retirement (1965). He was one of the most prolific .

His life served as an excellent example to illustrate that a person who wants to work after his retiring can do very well. During the years of retirement Professor GREGUSS wrote 61 articles. At present two of his major works are still in print.

From the beginning of the 1930's, he took up the research of extinct plants (fossils). His monograph entitled "Közép-Európai lomblevelű fák és cserjék meghatározása szövettani alapon" was published in 1947 and it made his name known among phytotomists.

Then his attention turned to the research of the structure of the gymnosperm and he completed his monograph entitled "Identification of Living Gymnosperms on the Basis of Xylotomy" in 1955 which contains 360 gymnospermous plants. This monograph has become known all over the world.

As the author of several books and papers Professor PÁL GREGUSS is known as a xylotomist for biologists in many countries.

During the four decades he spent as a professor he trained generations of teachers and researchers. They are now professors and highly qualified researchers taking a prominent part in the Hungarian world of science.

The main object of the lectures of Professor GREGUSS was to present the theory of evolution. In this excellent lectures he suggested love for nature, life and humanity. In this context he often spoke of the three greatest gifts of life namely about knowledge, love and health. He had a great share in all the three. His students loved him and attended his lectures with pleasure. He dealt with the flora of the different geological epochs in a clear and suggestive way.

Beside being a scientist and lecturer he was a man of public as well. He became the President of the Teachers' Trade Union formed in 1945 in Szeged. He was elected to be the Rector of our University in 1956.

He was rewarded many times in recognition of his scientific and educational work, the most important being the Kossuth Prize and the Order Decorated with the Flag of the Hungarian People's Republic.

The death of Professor GREGUSS is a grievous loss for both our university and Hungarian botany.

His name is enshrined in our memory for ever.

Editor's Note:

As for the scientific publications of Professor GREGUSS see our next number. Due to the great number of writings the material is being prepared for the press.

DR. S. GULYÁS

Doctor honoris causae

DR. A. A. KRASNOVSKY, Member of the Academy of Sciences of USSR, Head of the Photobiological Laboratory at the Bach Biochemical Institute.

Scientific Degrees

Doctors of Biological Sciences obtained by:

DR. L. OROSZ (Department of Genetics) with the dissertation "The moderate bacteriophage molecular genetics of Rhizobium meliloti 16–3", and by.

DR. F. ZSOLDOS (Department of Plant Physiology) with the dissertation "The effect of environmental factors on the ion uptake of plants".

Candidate of Biology:

DR. L. ERDÉLYI (Department of Comparative Physiology), obtained with the dissertation "Comparative physiological relations of the functioning of chemical synapses".

Appointment

The Ministry of Cultural Affairs has appointed DR. F. KEVEI to Assistant Proffessor at the Institute of Microbiology.

Retirings

DR. B. MAGDOLNA VARGA, scientific advisor (Department of Plant Physiology) and DR. H. MÁRIA MÉSZÁROS, Assistant Professor (Department of Genetics).

VARIA

The 9 biological departments have been integrated into a department group (Zoology, Biophysics, Biochemistry, Anthropology, Genetics, Microbiology, Plant Physiology, Botany and Comparative Physiology), of which DR. L. SZALAY, Professor, head of the Department of Biophysics, became the director.

Index

Felelős kiadó; Farkas Gyula
84-2269 — Szegedi Nyomda — Felelős vezető; Dobó József igazgató
Készült monó szedéssel, íves magasnyomással, 18,5 (A/5) ív terjedelemben,
az MSZ 5601—59 és 5602—55 szabvány szerint